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Application Methods of Paclobutrazol in Mango Cvs & Its Residual Effect in Leaf, Fruit and Orchard Soil

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ABSTRACT

Paclobutrazol ([2RS, 3RS] -1 - [4-chlorophenyl] - 4, 4-dimethyl-2-[1, 2, 4-triazol-1-yl] pentane-3-ol) belonging to the class of growth regulators with the triazole chemical group, with hazard classification of class III. Paclobutrazol remained in the soil up to 11 months when it was applied to soil and three months in soil drench application without soil cover. The soil drench application of Paclobutrazol at the rate of 1.0 gram and 0.5 gram per canopy diameter applied in the month of 15 October and 15 September 1997 and 1998 respectively for three consecutive years and the residual effects were observed for three more subsequent years. Soil drenching was most effective in suppressing shoot elongation. The level of the residues was high in the leaves subjected to soil drench application. Residual effect was found at high amount in leaf receiving both soil drench application and at low quantity in the soil methods but in all treatments but chemical residue was also detected in the mature fruit. Paclobutrazol was effective in inhibiting shoot elongation and leaf expansion. The total flowering percentage in trees at all paclobutrazol application was higher than that of the control tree, but the applied paclobutrazol had positively effected on fruit set and yield. The PBZ residue in fruits including seed was always less than 0.01 mg/kg, which was much lower than level of accepted (by FOA) for consumption of Mango fruits (0.01 mg/kg). However the employing bioassay of PBZ residue obtained 0.00146 to 0.06100mg/kg in the year 1997-98.

Keywords: *Mango, Mangifera indica, paclobutrazol, shoot vigour, fruit yield, fruit quality*

INTRODUCTION

The improvements in crop productivity in modern agricultural systems are increasingly dependent on manipulation of the physiological activities of crop by chemical means paclobutrazol is a plant growth regulator which has been used in fruit tree crops to control vegetative growth and to induce flowering. Paclobutrazol can be applied to mango trees as by soil drenching. In previous reports, it was indicated that paclobutrazol can be applied by trunk injection which also resulted in the reduction of shoot growth in many crops. The objectives of this study were to determine the effectiveness of application methods of paclobutrazol on the vegetative and reproductive growth of mango trees, and to detect its residues in leaves, fruit and soil. Control of vegetative vigour with simultaneous promotion of flowering is important for enhancing the production efficiency of mango orchards (Iyer and Kurian, 2002). Although the direct effects of paclobutrazol (PBZ), on the growth and flowering of mango have been well documented (Kulkarni, 1988; Kurian and Iyer, 1993 a, b, c; Burondkar and Gunjate, 1991) and many mango orchardists in western and southern parts of India have adopted application of PBZ for higher mango production, there is little published information on long term effects of its continuous application as well as residual influence of the chemical on growth, yield and fruit quality of mango in the years following the discontinuation of its application. Chemical parameters of fruits such as TSS and acidity were not affected by the treatments but average weight of a fruit was less in the case of PBZ treatments.

Residual influence of this chemical, when applied as soil drench, persisted in the three years following the discontinuation of application for three consecutive years, indicating the scope for skipping the application of PBZ or tapering down its dose after three years of its continuous application.

From the results of this study, application of paclobutrazol at 1.0 gram per canopy diameter per tree as soil drench for three consecutive years appears to be most appropriate for mango cultivars Dashehri, langra, chausa and Fazri trees in the age group of about 15 to 25 years compare to lower doses and control.

MATERIALS AND METHODS

Twenty years old trees of the mango cultivar orchard at Horticulture Research Centre, Patherchatta, GBPUA&T-Pant Nagar were used in this study during PhD programmed. On 01 August, 1997-99, twenty uniform trees were treated with paclobutrazol by soil drenching at a level of 1.0 and 0.5 gram per canopy diameter with control into around a trunk basin. The experiment was conducted in a two factorial randomized design with four replications with one tree as a replicate. After the treatments, the leaf area and shoot elongation were measured at 15 days intervals on 20 shoots per tree. The latest mature leaf was used for the leaf area measurement. Two months after paclobutrazol application, flowering was induced by paclobutrazol treatment; the percentage of flowering was determined by counting the number of inflorescences in 20 shoots per treatment. The number of fruit set in an inflorescence was counted in randomly selected inflorescences for each treatment, using 20 inflorescences per tree. Immediately after 15 days the application of paclobutrazol, 100g of fresh leaf and 100g soil to a 30 cm depth below the ground level from four sites under the canopy were collected for evaluating the amount of paclobutrazol residues for each tree.

At the fruit maturity, 100g of pulp was collected for evaluating the paclobutrazol content in the fruit. The level of paclobutrazol in the leaf, fruit and orchard soil was determined by bioassays. The plant samples were homogenized in 95% methanol with a commercial blender by a modified method. The extract was filtered under vacuum conditions and the filtrate was dried at 60˚Ċ in a water bath. Then, the residues were dissolved in 10ml of 18% methanol and mixed with 60ml of 99.5% methylene chloride in a separating funnel for different bioasseyed.

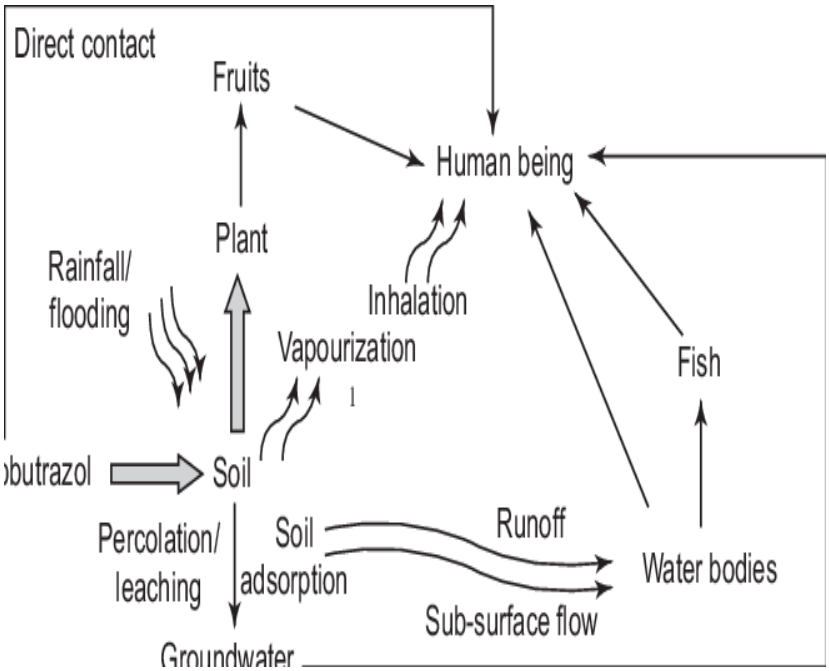

Figure1. *Schematic presentation of paclobutrazol residue movement in the environment*

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non-polar mobile phases such as heptanes are used. The mobile phase

may be a mixture, allowing chemists to fine-tune the bulk properties of the mobile phase.

Extraction

The methodology employed was an adoption of Mauk et al, (1989) with certain modification after 100 g of fresh plant material (fruit skin, pulp and seed), leaf and soil sample were extracted with chilled 80% Methanol for 5 minute with Ramie mixture. The Methenolic extracts of each sample were pooled separately and reduced to aqueous state in vacuum at 35˚C temperature and pH adjust of the aqueous fraction was raised 11.0 with 1 M NaOH and extracted 4 times with Methylene Chloride. The Methylene Chloride was evaporated to dryness in vacuum and residue was taken up in Methanol for estimation of Paclobutrazol.

Paper Chromatography

Ascending paper chromatography was carried out for the separation of PBZ from soil, leaf peel, mesocarp and seed extract. The solvent system employed was Acetone: Hexane (1:2 v/v), the extract was taken in Methanol and streaked as 5mm band across a What man No. 1 paper strip and chromatograms were developed in glass cylinder as described by Nisch (1955). The solvent was allowed to ascend up to 20cm height on the paper and dried in dark each dried chromatograms was cut into 10 equal segments and was kept in separate test petriplates (9 cm diameter). The elutes of the chromatographic segments in petriplates were bioassay.

Thin Layer Chromatography

Thin layer plates of Silica gel G (60 Mesh) of 0.25 mm thickness were prepared on glass plates (20x20 cm). The plates were activated at 1200 C for one hour before use. The authentic sample of PBZ in different amount were load as a spot on thin layer plates employing solvent system Hexane: Acetone, (v/v) the absolute from

Development chromatograms were removal in 10 segments and used for bio assay Corn Root Curvature Test.

Bioassay of Paclobutrazol

Corn Root Curvature Test PBZ

Paclobutrazol activity was tested in Corn Root Curvature Test. Curvature Test as described by Curtis (1985). Five ml of test solution was paused in each petriplates containing what man No. 1 filter paper disc (9.0cm). The seeds of Corn Cvs. Sweta presoaked and thoroughly washed were arranged introversely on the Periphery of filter paper or segment of chromatogram in each petriplates. The petriplates were kept at 25+10 C for 72 hours in dark. The number of roots having more than 900 curvatures was counted after 72 hours.

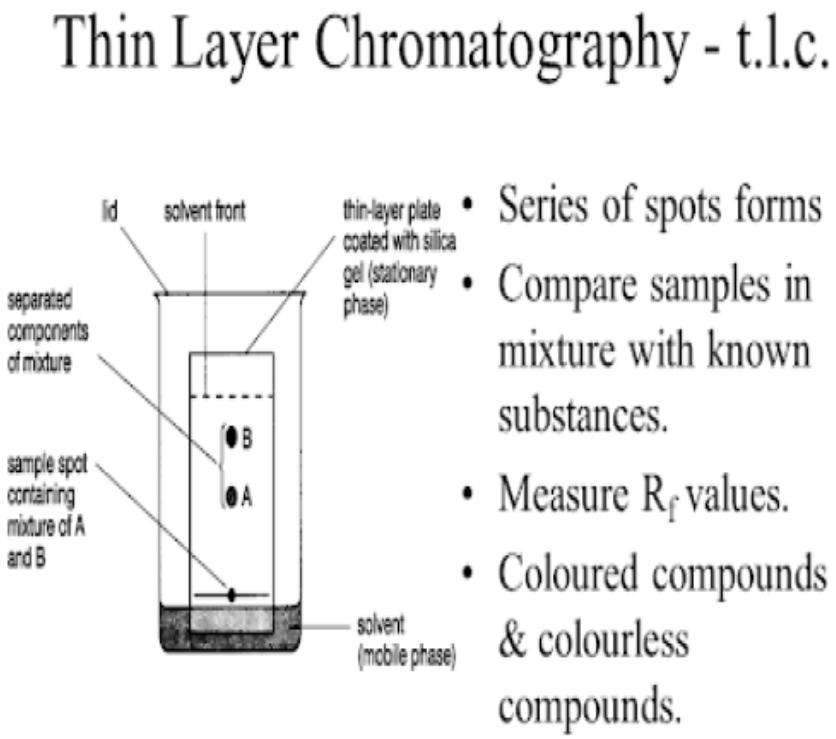

Figure2. *Parts of Thin Liquid Chromatography*

Table1. *Paclobutrazl residue level in Leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Treatments	Date of Observation	Sampling	Quantity of Extracts (ul) load on paper chromatography	Quantity of PBZ in mg/Kg fresh weight
Dashehari Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Leaf	100 ul	0.00000 0.02618 0.04213
Langra Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Leaf	100 ul	0.00000 0.02618 0.04213
Chausa Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Leaf	100 ul	0.00000 0.02597 0.04113
Fazli Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Leaf	100 ul	0.00000 0.02213 0.04012
CD at 5%, Treatment, Variety, Interaction	-	-	-	-

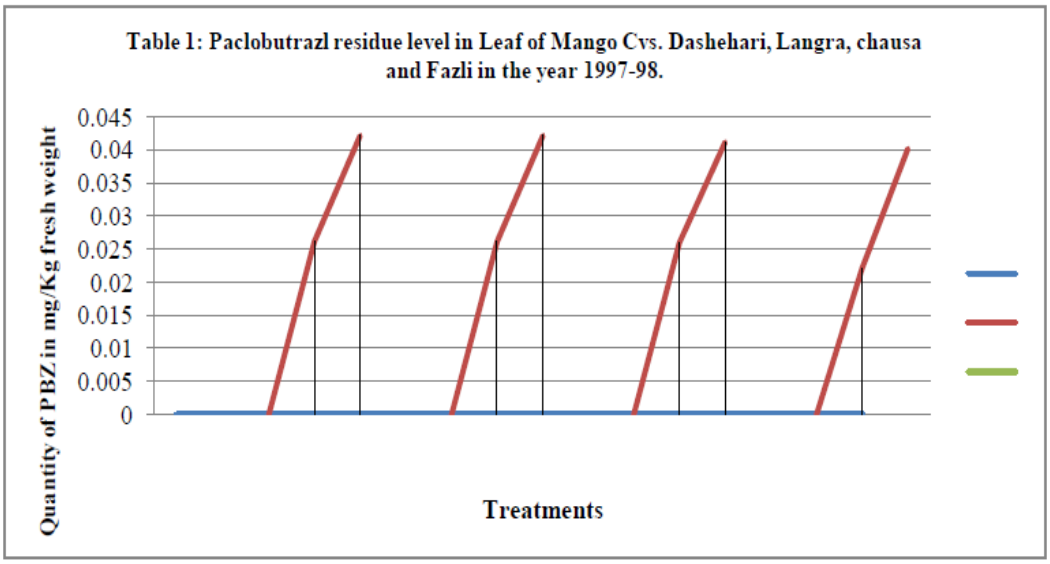

Figure3. *Paclobutrazl residue level in Leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Table2. *Paclobutrazl residue Soil in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Treatments	Date of Observation	Sampling	Quantity of Extracts (ul) load on paper chromatography	Quantity of PBZ in mg/Kg fresh weight
Dashehari Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Soil	100 ul	0.00000 0.03298 0.06101
Langra Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Soil	100 ul	0.00000 0.03298 0.04170
Chausa Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Soil	100 ul	0.00000 0.01967 0.04172
Fazli Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Soil	100 ul	0.00000 0.01607 0.02671
CD at 5%, Treatment, Variety, Interaction	-	-	-	-

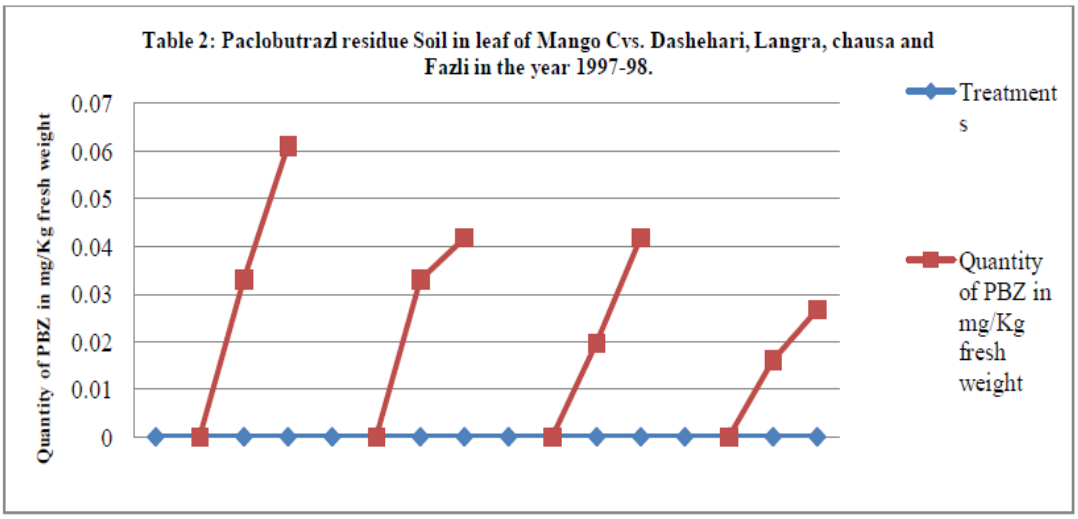

Figure4. *Paclobutrazl residue Soil in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Table3. *Paclobutrazl residue Peal in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Treatments	Date of Observation	Sampling	Quantity of Extracts (ul) load on paper chromatography	Quantity of PBZ in mg/Kg fresh weight
Dashehari Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Peal	100 ul	0.00000 0.01233 0.02103
Langra Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pea	100 ul	0.00000 0.00111 0.00162
Chausa Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pea	100 ul	0.00000 0.00123 0.00290
Fazli Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pea	100 ul	0.00000 0.00114 0.00816
CD at 5%, Treatment, Variety, Interaction	-	-	-	-

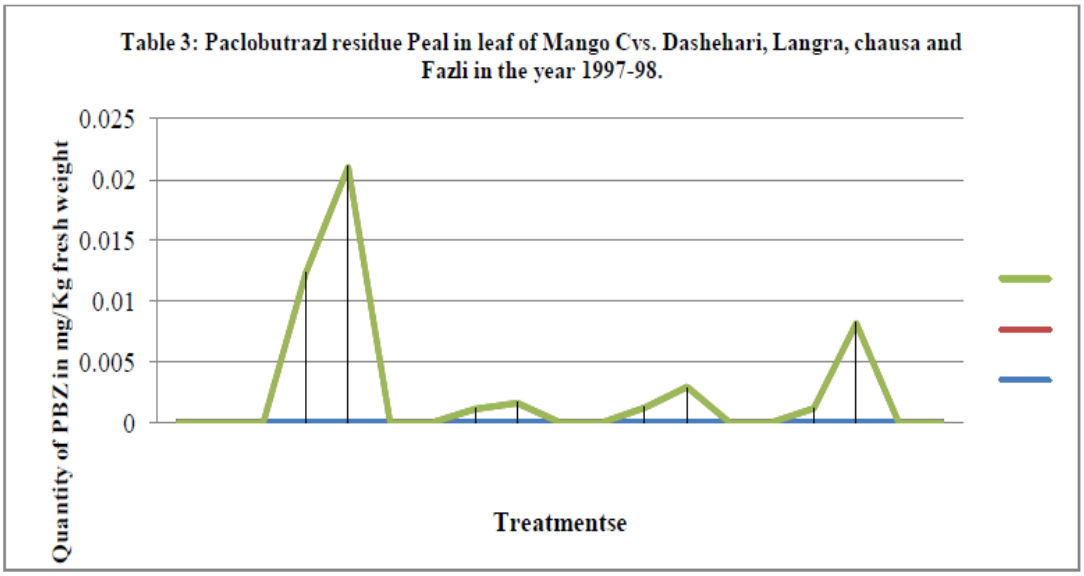

Figure5. *Paclobutrazl residue Peal in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Table4. *Paclobutrazl residue Seed in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Treatments	Date of Observation	Sampling	Quantity of Extracts (ul) load on paper chromatography	Quantity of PBZ in mg/Kg fresh weight
Dashehari Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Seed	100 ul	0.00000 0.00152 0.00268
Langra Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Seed	100 ul	0.00000 0.00147 0.00270
Chausa Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Seed	100 ul	0.00000 0.00127 0.00267
Fazli Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Seed	100 ul	0.00000 0.00147 0.00321
CD at 5%, Treatment, Variety, Interaction	-	-	-	-

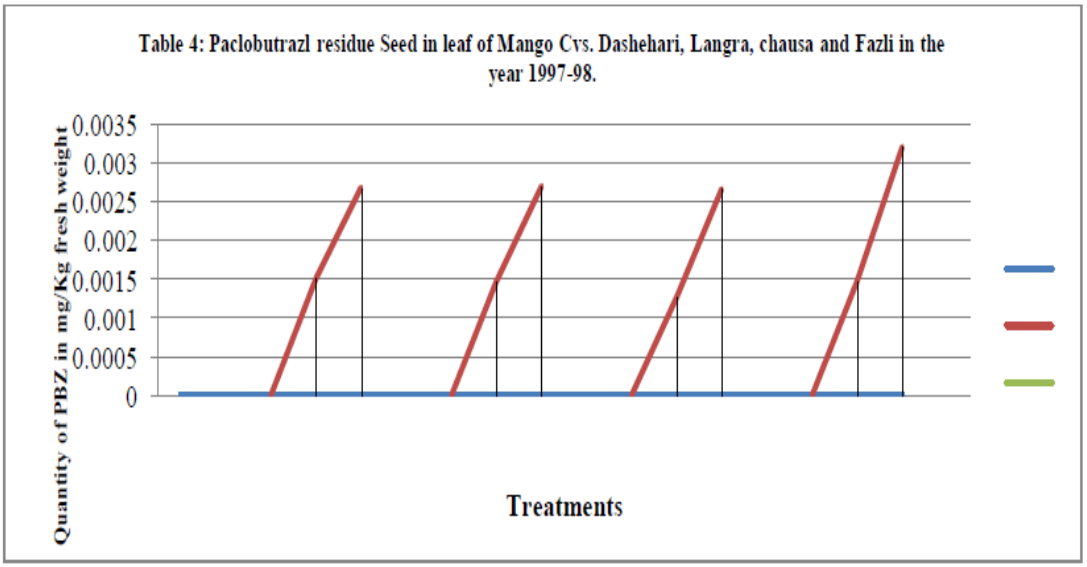

Figure6. *Paclobutrazl residue Seed in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Table5. *Paclobutrazl residue Pulp in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

Treatments	Date of Observation	Sampling	Quantity of Extracts (ul) load on paper chromatography	Quantity of PBZ in mg/Kg fresh weight
Dashehari Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pulp	100 ul	0.00000 0.00812 0.01462
Langra Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pulp	100 ul	0.00000 0.00121 0.00146
Chausa Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pulp	100 ul	0.00000 0.00100 0.00153
Fazli Control 0.5 gram PBZ in canopy diameter. 1.0 gram PBZ in canopy diameter.	15 Dec/15 Jan	Pulp	100 ul	0.00000 0.00127 0.00136
CD at 5%, Treatment, Variety, Interaction	-	-	-	-

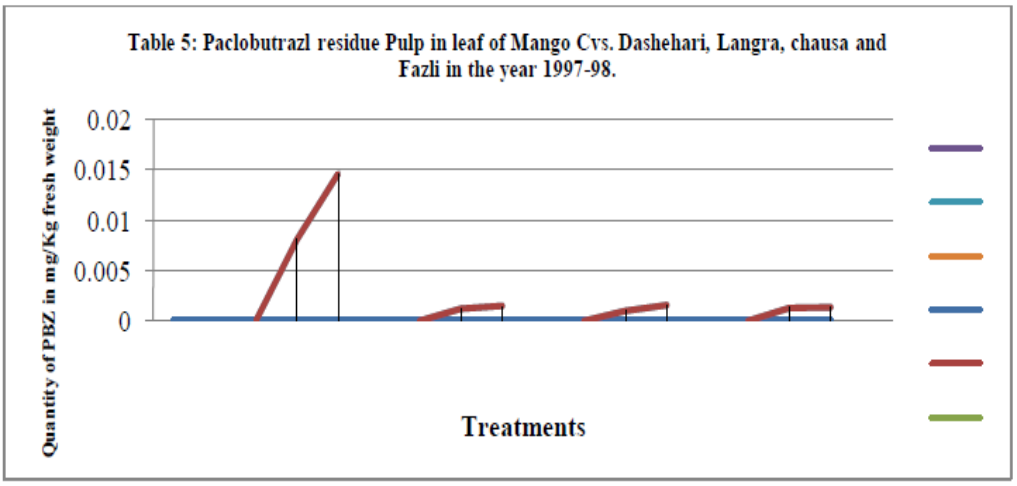

Figure7. *Paclobutrazl residue Pulp in leaf of Mango Cvs. Dashehari, Langra, chausa and Fazli in the year 1997-98*

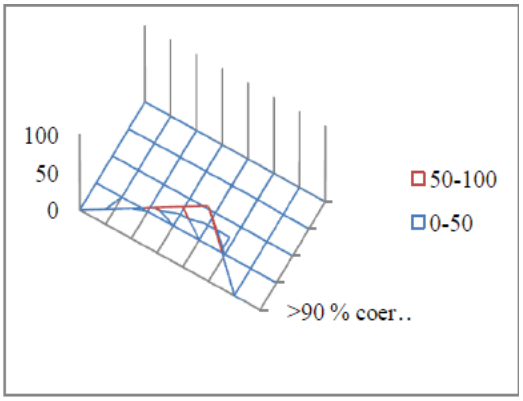

Figure8. *>90 % root curvature test For Paclobutrazol (CD at 0.5% 18.1)*

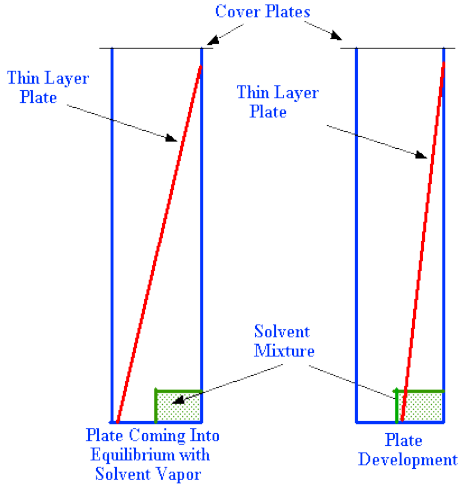

Figure9. *Front View of Thin Layer Chromatography*

After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are treated with a phosphor, and dark spots appear on the sheet where compounds absorb the light impinging on a certain area. Chemical processes can also be used to visualize spots; anisaldehyde, for example, forms colored adducts with many compounds, and sulfuric acid will char most organic compounds, leaving a dark spot on the sheet.

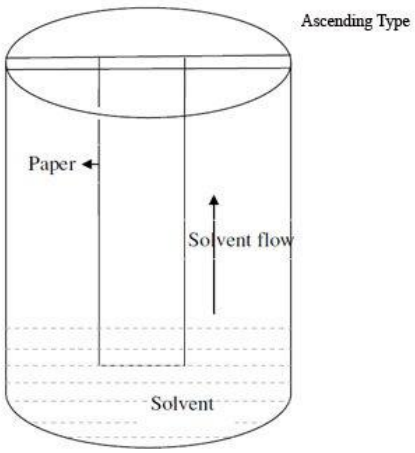

Figure10. *Asending Paper Chromatography*

To quantify the results, the distance traveled by the substance being considered is divided by the total distance traveled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase.) This ratio is called the retardation factor (R_f). In general, a substance whose structure resembles the stationary phase will have low R_f, while one that has a similar structure to the mobile phase will have high retardation factor. Retardation factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, chemists usually apply a sample of a known compound to the sheet before running the experiment.

What Is Paper Chromatography?

Paper chromatography is one of the types of chromatography procedures which run on a

piece of specialized paper. It is a planar chromatography system wherein a cellulose filter paper acts as a stationary phase on which the separation of compounds occurs.

Principle of Paper Chromatography

The principle involved is partition chromatography wherein the substances are distributed or partitioned between liquid phases. One phase is the water, which is held in the pores of the filter paper used; and other is the mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in stationary phase) and mobile phase solvents during the movement of mobile phase under the capillary action of pores in the paper.

Analytical Method of Paper Chromatography

are used to separate colored chemicals or substances. It is primarily used as a teaching tool, having been replaced by other chromatography methods, such as thin-layer chromatography. A paper chromatography variant, two-dimensional involves using two solvents and rotating the paper 90° in between. This is useful for separating complex mixtures of compounds having similar polarity, for example, amino acids.

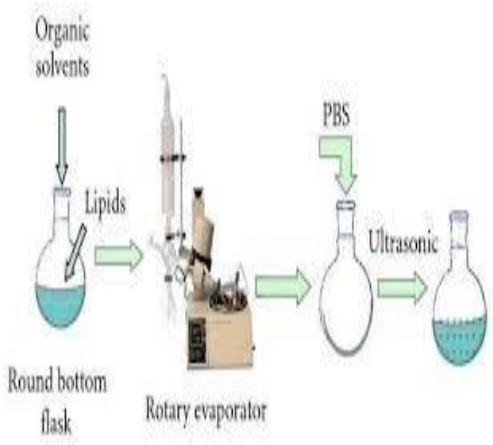

Figure11. *Rotary Evaporator and its Methods*

The setup has three components. The mobile phase is a solution that travels up the stationary phase, due to capillary action. The mobile phase is generally mixture of non-polar organic solvent, while the stationary phase is polar inorganic solvent water. Here paper is used to support the stationary phase, water. Polar water molecules are held inside the void space of cellulose network of the host paper. Difference between TLC and paper chromatography is that stationary phase in TLC is a layer of adsorbent (usually silica gel, or aluminum oxide), and stationary phase in paper chromatography is water. The principle can also be adsorption chromatography between solid and liquid phases, wherein the stationary phase is the solid surface of the paper and the liquid phase is of the mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography, i.e., partitioned between to liquid phases. Paper chromatography is specially used for the separation of a mixture having polar and non-polar compounds.

Separation of Paclobutrazol

It is used to determine organic compounds, biochemicals in urine, etc. In the pharmacy sector, it is used for the determination of hormones, drugs, etc. Sometimes it is used for evaluation of inorganic compounds like salts and complexes.

Ascending Paper Chromatograph

Paper chromatography is one method for testing the purity of compounds and identifying substances. Paper chromatography is a useful technique because it is relatively quick and requires only small quantities of material. Separations in paper chromatography involve the same principles as those in thin layer chromatography, as it is a type of thin layer chromatography. In paper chromatography, substances are distributed between a stationary phase and a mobile phase. The stationary phase is the water trapped between the cellulose fibers of the paper. The mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it. Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase. Paclobutrazol residues in mango fruits for analysis of HPLC/Flow This work was developed in to b 5 steps: (a) selection of growth regulator for mango samples, (b) optimization of chromatographic conditions, (c) considering factors that supplies better resolution and less time of analysis, (d) development of method to analyses paclobutrazol in mango fruit, (e) determine paclobutrazol in mango samples by Thin Liquid chromatography/Paper chromatography.

The Analysis of PBZ Residues

The analysis for determination of residues of PBZ in chromatography has been applied in

studies of dissipation. For analysis we used standard analytical paclobutrazol (Sigma) and the reagents and solvents: acetonitrile solution HPLC grade, ultrapure water (70:30), dichloromethane HPLC grade, anhydrous sodium sulphate PA and methanol HPLC grade, the steps as: Weigh 10 g of mango samples into a tube for centrifugal, add 30 mL of solution of acetonitrile: water (70:30); shake in Turrax for 2 min; centrifuge at 4000 rpm for 15 min, the supernatant transferred to round-bottomed flask, repeat the procedure in item 2 to 6, transfer the extract to the separation funnel, add 30 mL of dichloromethane, shake for ± 1 min, filter the organic phase in a filter paper containing ± 5 g of anhydrous sodium sulphate PA, repeat the procedure of item 9 to 11; evaporate the sample to dryness, resuspend in 10 mL of methanol in concentrator tube and for quantification of the PBZ samples of leaf, soil and pulp was used external standard method using standard curve obtained under the same conditions of the sample. It was used as control sample of the fruit without fortification (addition of the standard). For the blank, was followed by the extraction procedure, using only the solvents. From the samples were not detect residues of PBZ within the method and conditions of study, as shown in the chromatogram.

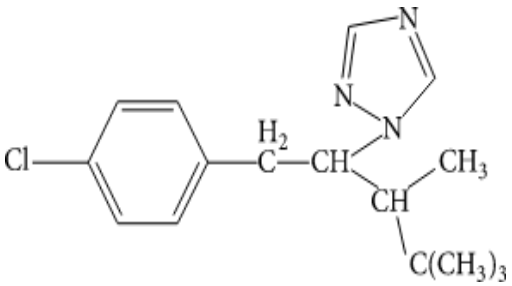

Figure12. *Paclobutrazol ([2RS, 3RS] -1 - [4-chlorophenyl] - 4, 4-dimethyl-2-[1, 2, 4-triazol-1-yl] pentane-3-ol*

Results and Discussion

Paclobutrazol is one of the extensively used plant growth regulators used especially in mango fruit. Primarily, the role of paclobutrazol is to inhibit gibberellins synthesis and promote flowering and fruiting. Thus, the vegetative growth reduces and the assimilates are transferred to reproductive organs and the yield potential increases.

Flowering Initiation

Flower initiation is very important because it is the first step towards attaining fruit. Recent trials have clearly shown that while the extent (quantity) of flowering affects yields, time of flower emergence has a significant influence on time of fruit maturity. Early flowering clearly resulted in early fruit maturity. Unfortunately, our commercial variety does not flower regularly year after year. Flowering is also staggered, leading to considerable variation in fruit maturity. The induction of regular, profuse, early and uniform flowering will undoubtedly ensure higher yields and better returns to the grower.

The Effects of Paclobutrazol

One method to manipulate flowering is to use the plant growth regulator **paclobutrazol**. The post- harvest application of a small amount of paclobutrazol to the soil significantly promotes flowering and fruiting in the following year. Trials over the last two years have shown the following benefits from the treatment: **1.)** A significant increase in flowering leading to increased yields. **2.)** The early flowering considerably enhanced fruit maturity. Treated trees flowered three to four weeks early, which reduced the time to fruit maturity by at least two weeks. **3.)** Visually, the fruit developed a better external colour.

The Use of Paclobutrazol

How does paclobutrazol act? Available evidence strongly suggests that flower initiation depends on the presence of an unknown flower promoting factor or factors synthesized in the leaves. At the same time, there are other factors in the shoots which work against the flowering factor or other complex factors. It is believed that a group of plant hormones called gibberellins act as inhibitors to flowering. When paclobutrazol is applied to the soil, it moves up through the roots into the shoots and, due to its anti-gibberellins properties, blocks the synthesis of flowering inhibitors, thereby allowing the flower-promoting factor(s) to work.

How and when to Apply Paclobutrazol?

The application of paclobutrazol to soil as a drench around the tree trunk (collar drench) is the most effective method, as it ensures proper uptake by the tree. The required quantity is mixed in approximately one liter of water and poured onto the soil around the trunk in a circular band. In the Top End, the ideal time to apply paclobutrazol is from soon after harvest to early 15 October. In dry conditions, a light irrigation is recommended after application. Foliar sprays have been ineffective.

At What Age should Trees be Treated and when should Treatment be Repeated?

The size of trees at first application is important. This depends on the age of the trees and the spacing between them. Apart from promoting flowering, paclobutrazol also restricts tree vigor. Trees should therefore be allowed to develop a good canopy before treatment commences. In high tree density situations with closer spacing, it is recommended to apply paclobutrazol early when trees are about three years old. However, when trees are spaced farther apart, say 10 m, early application with paclobutrazol will reduce canopy size and the fruit bearing area. In such a situation, treatment can commence when trees are about five years old. Tree size and canopy fill are important considerations.

Large trees, especially seedling trees, respond more slowly than young, bearing, grafted trees. The dosage required also varies between cultivars. Florida cultivars, such as Irwin, Glen and Tommy Atkins require a lower dosage than Kensington Pride. At excessively high dosages, flower and shoot compaction can lead to increased infestation by caterpillars. If such compaction occurs, the dosage of repeat applications should be reduced., Two sprays of potassium nitrate at 4 g/L at ten-day intervals, commencing at signs of flower bud burst, were found to minimize panicle compaction. However, compaction is best prevented by using an optimum dosage. If you are not sure about dosage and/or if your trees are ready for the treatment, seek expert advice.

Tree Health and Nutrition

Any treatment that leads to increased production should be supported by good management to maintain tree healthy. This includes nutrition, irrigation, control of pests and diseases, pruning and skirting. It is desirable to prune and skirt trees after harvest and before the treatment. Unhealthy and weak trees should not be treated with paclobutrazol.

Effect on Growth and Development

Leaf Area

The leaf area was not significantly different among the treatments up to 12 months after paclobutrazol application. However, the leaf area of the soil drench application trees was reduced to 31% of that of the control only at two months after the treatment.

Shoot Growth

There was significant reduction in length of new shoots produced following PBZ application, the effect being more marked with soil application than foliar spray and increasing with the dose of the chemical within each method of application as per the earlier findings (Burondkar and Gunjate, 1991; Kurian and Iyer, 1993a). PBZ is a known inhibitor of gibberellin biosynthesis (Anon, 1984) and therefore lower gibberellins levels resulting from its application might have retarded the shoot elongation.

The inhibitory effect of PBZ on shoot elongation slowly dissipated once its application was discontinued and differences in shoot length were not statistically significant during 2000 to 2002, though the shoots on treated trees remained shorter than those on control trees. This reduction in shoot elongation serves to control excess vegetative vigour and thereby to restrict the canopy size of mango trees, which would facilitate easier orchard management practices as well as planting mango trees at higher densities than the conventional one. Flowering Enhanced proportion of flowering shoots through a reduction in proportion of vegetative and dormant shoots, was a striking response to PBZ treatments, which was more pronounced with soil application rather than foliar spray.

This effect was quite discernible in all the years of study except during the year 1999 when the natural flowering was high with even the control plants putting forth panicles in 91% of their shoots and continued in years after the treatment was stopped, though statistically not significant, particularly in the case of soil treatments. Thus PBZ, especially as soil drench, was especially effective in enhancing flowering during years of sparse natural flowering and the residual effect of the chemical in this regard may persist for two to three years after application is stopped. Such enhanced flowering of mango trees following PBZ treatments has earlier been reported by Kurian and Iyer (1993b), Burondkar and Gunjate (1991) and Kulkarni (1988). The present study indicates the scope for skipping the application of the chemical after a few years of its continuous application or tapering down its dose, especially during the years when good flowering is expected, while continuing to get the beneficial influence on flowering. Shoot growth response to paclobutrazol was different between the application methods.

The shoot length at six and seven months after the treatment decreased in the soil drenching treatment. Foliar spray, foliar spray and covering of soil with a plastic sheet, resulted in the reduction of shoot growth at seven months after the application. These results indicate that the shoot reduction effect of paclobutrazol persists longer with soil treatment than with foliar application. Similar results were reported for apple trees.

Fruit Yield

Fruit yield in terms of number and weight of fruits per tree increased with application of PBZ and this was more striking in the case of soil application than foliar spray. This effect dissipated in the year following withdrawal of PBZ treatment in the case of foliar spray and in the second year following withdrawal of PBZ treatment in the case of soil application at lower dose while the effect continued to manifest in the case of soil application at higher dose. Though beneficial effects of PBZ in enhancing fruit yield of 'Alphonso' mango have earlier been documented by Kurian and Iyer (1993c) and Burondkar and Gunjate (1991), the present study reveals that the soil application of PBZ can be temporarily withdrawn for three years or so after three years of continuous application without a reduction in fruit yield. PBZ alters the source-sink relationships in mango to support fruit growth with fewer leaves and lesser leaf area (Kurian et al, 2001), which explains the enhanced fruit yield with lesser vegetative growth.

Percentage of Flowering

The percentage of flowering after 75 days of any of the paclobutrazol treatments was higher compared with that of the control. The percentage was highest in the case of soil drenching and lowest in the case of lower doses. In pear, the yield of the trees treated with paclobutrazol was higher than that of the unsprayed trees. The results in this study suggest that treatment combining paclobutrazol and thiourea is an effective method to increase production in mango trees. Apple tree treated with paclobutrazol showed an increase in the carbohydrate content in the uppermost leaves; all stem portions and especially in fibrous roots. The content of total nonstructural carbohydrates per plant markedly increased by paclobutrazol treatment because more assimilates was partitioned into stems, leaves and roots6). The increase of the rate of flowering with paclobutrazol in mango might be. caused by the increase in the carbohydrate level.(Data not shown)

Fruit Set

The number of fruit set in mango tree can be determined by counting the number of fruits in the inflorescence. In this experiment, the number of fruits per inflorescences was 6, 8, 10 and 9 fruits in the case of foliar spray, foliar spray and covering soil with a plastic sheet, soil drenching and trunk injection, respectively. Fruit set was higher in all of the paclobutrazol treatments than in the control where 5 fruits were set. . (Data not shown)

Fruit Quality

There was no appreciable influence of the treatments on chemical parameters of fruit quality such as total soluble solids and acidity, but average weight of a fruit reduced as a result of PBZ treatment, more so with the soil application. Almost similar trend was observed by Kurian and Iyer (1993c) as a direct response to PBZ application. The influence of PBZ on fruit size continued even during three years following withdrawal of its application in the present study. Incidence of spongy tissue disorder in fruits of 'Alphonso' mango was unaffected by different PBZ treatments, but ripening of fruits harvested at full maturity was delayed by PBZ as indicated by number of days taken for ripening of 50% of the harvested fruits. This effect was however not statistically significant in the years after application of the chemical was stopped. . (Data not shown)

Residues of Paclobutrazol

The residual analysis of Paclobutrazol in soil, leaves and fruits describes as follows:-

Residues in Orchard Soil

Paclobutrazol was detected in orchard soil subjected to soil drenching and foliar spray. The residues persists longer in the case of soil drenching (11 months) than in the case of foliar spray (6 months). In soils with a high cation exchange capacity like in this experiment, the chemical was absorbed by the clay and organic particles where it persisted for a longer period of time. (Table-2)

Residues in Leaves and Mature Fruits

Paclobutrazol was detected in the leaves of all trees in all the four application methods. In case of foliar application, the chemical residues in

the leaves persisted for 3 months and the chemical was detected again after 7 and 8 months. Re absorption of the chemical from the other parts of the tree might occur, due to rainfall in the rainy season that usually begins from May in this area. For the trunk injection, the chemical was detected in the leaves one month after the injection and the residues in the leaves persisted for two months. In the soil-treated trees, the residues were detected in the leaves two months after the application and persisted for two months. Although the residues remained much longer in soil, they were not detected in leaves four months after the application. The absorption of the chemical seems to be slow and limited, once it is applied to soil. In all the treatments, the residues of paclobutrazol were hardly detected in mature fruits. This finding suggests that the various application methods of paclobutrazol examined in this experiment can be used for mango production in terms of food safety. However, the effect of the residues in soil and the outflow of the chemicals to the surrounding area on the environment also should be assessed thoroughly. Based on the effectiveness for retarding vegetative growth and enhancing flowering, it is concluded that soil drenching is the most practical method of paclobutrazol application in off season mango production in Thailand. (Table-1)

Residual Analysis through HPLC

According to Lanças (2004) and Green (1996) a simple way to verify the selectivity of the chromatographic method is to observe the presence of peaks in the retention time of analyte, injecting the blank with the same matrix to be analyzed. It should be noted the absence of peaks near the retention time. Retention time (RT) of PBZ standard was 6.42 minutes. To prove the selectivity of the method, extractions were made of fruit, without the addition of standard and injected in triplicate and is not observed interfering peaks near the retention time of default. Neither the matrix effect was observed. As the repeatability of the chromatographic method, standard samples were injected at the same concentration and evaluated, obtaining 0.017 as standard deviation and coefficient of variation (%) equal to 0.2654. Linear range was between 0.75 – 3.0µg L-1, equation of a straight line was y = 80,734x + 9,1331 and the correlation coefficient obtained was 0,9942. Assessing the recovery (%) of the method after the analysis of spiked (1.0, 1.5, 2.0 µg L-1), obtained as a result the values of recovery samples, 86.58, 79.33 and 92.11% respectively. In recovery, the values found are in the range of 70 to 120% for all products screened. For quantification of the PBZ samples of pulp was used external standard method, using standard curve obtained under the same conditions of the sample. The calibration curve was established correlating standard versus peak area, in concentrations 0.75, 1.0, 1.5, 2.0, 3.0 µg L-1. It was used as control sample of the fruit without fortification (addition of the standard). For the blank, was followed by the extraction procedure, using only the solvents.

Table6. *Recoveries of the Paclobutrazol from Fortified mango fruit control sample*

Fortification Concentration in µg/mL	Replication	Recovery in
0.3	R1	89
	R2	86
	R3	87
	R4	89
	R5	91
Mean	-	88.4
RSD	-	1.51

Residual analysis through HPLC

CONCLUSION

A sample for the determination of Paclobutrazol residues in fruits was developed. The sample was extracted with 80% methanol-water solution, then was purified by C 18 column and detected in 220 nm with a DAD detector. Linear ranges of three plant growth regulators were in the range of 0. 5 ~ 10µg / mL with correlation coefficients more than 0.999. The minimum detection limit of Paclobutrazol and were 0. 0. 04 mg / kg respectively. Data further suggest that in ripe fruits mesocarp the Residue level decreased to a half to that of a mature fruits mesocarp and higher residue in mesocarp seed and episarp of the fruit in Paclobutrazol treated trees than that of its doses. (Table 4,& 5) Higher amount of Paclobutrazol residue in the peal was also obtained in apple (Anon,1986) and Paclobutrazol residue in soil was also more in such trees. Some Paclobutrazol treated trees have high Paclobutrazol residue in seeds shows vivipary and deformed seedling after germination of these similar to that of Paclobutrazol treated seeds (Tripathi, 1993). Thus Paclobutrazol appears to cause deformity to Mango seedling similar to ethylene (Tripathi 1993) and malformin (Singh, 1996).

The fruits under different treatment were separated into epicarp, mesocarp and seeds immediately after harvest and kept in 80% chilled Methanol and another set of fruits were allowed to ripen at ambient temperature (30+30 C) and then the Paclobutrazol in mesocarp (pulp) was estimated at ripe stage of the fruits The data is further shows that trees, which were not treated with Paclobutrazol, did not show any Paclobutrazol residue in soil, leaf and different fruit parts on the paper chromatography. Data shows that equation Y- 26.74+ 4.15X obtained during the standardization of Corn Root Curvature bioassay for estimation of Paclobutrazol. For this purpose Paclobutrazol of this sample was extracted into dichloromethane fraction as described in material and method section.(Data not shown)

The dichloromethane fraction, equivalent to 1.0 g tissues was chromate graphed by the employing Hexane: Acetone solvent system and chromatograms were bioassay by the Corn Root Curvature Test. Paclobutrazol migrate to Rf 0.5-0.8 on paper chromatogram developed in Acetone: Hexane (1:2) solvent system. When Paclobutrazol was applied at later dates 15 g Oct. and 15 Sep. for two consecutive years @ 1.0 g PBZ/tree followed by 0.5 g PBZ/tree canopy diameter. The PBZ residue in soils, leaf and epicarp remains higher than the lower doses of PBZ data further show that PBZ residue in soils, leaf and epicarp was having direct effect of doses and date of application. Which was not further coupled with number of year PBZ was not applied in continuation. Similar to the PBZ residue obtained in soils, leaf, epicarp of the fruits, its residue in mesocarp and seeds followed similar trends. Paclobutrazol residue was maximum in soil, leaf apocarps followed by seed and mesocarp in that order.

The ripe pulp always contains PBZ However he level of PBZ residue in the soil was always higher which were treated with doses to shorter period in continuation. Extractor of seed from ripe fruits and observation on this viviparous nature further suggest that there was a story co-relation between the vivipary of seed and PBZ residue on seed and soil. Therefore suggest that PBZ not only induces early repining of fruits but also induces vvipary. However, PBZ content of fruit was use that Paclobutrazol can be estimated by Corn root Curvature test originally developed for estimation of malformed activity (Curtis, 1985), Which was later on found to be sensitive to Ethephon (ethylene) and Paclobutrazol.

The present investigation also shows that Corn Root Curvature bioassay increased linearity with the in concentration in test medium, since Corn Root Curvature bioassay has been reported to be sensitive to malformin and Paclobutrazol (Curtis, 1958, 1968, Kumar et al. 1987). Corn Root Curvature test can be used as a bioassay method for estimation of Paclobutrazol residue and this test appears to be ethylene only. Thus Presence of Paclobutrazol in soil, leaf and fruit confirm its role in reduction of shoot growth, increased flowering and fruiting as well as better TSS and sugar in fruits besides early maturity of fruits as reported in Mango by other workers (Reynold, 1981; Reynold et al.; 1992) the accepted Paclobutrazol residue in Mango fruit including seed was always less then 0.01 mg kg. Fruit which was match lower than the level of acceptable for consumption of Mango fruits the Paclobutrazol residues in obtained by employing bioassay method show maximum Paclobutrazol in the soil for longer similar results were also obtained in cherry orchard (Belmas, 1989). However employing bioassay of Paclobutrazol residue obtained 0.00146 to 0.06100 mg/kg in year 1997- 98(Table 1 to 5) and 0.00100 to 0.03210 mg/kg in 1998-99 (Data not shown).

Data further suggest that in ripe fruits mesocarp the Residue level decreased to a half to that of a mature fruits mesocarp. Higher amount of Paclobutrazol residue in the peal was also obtained in apple (Anon, 1986) and Paclobutrazol residue in soil was also more in such trees. Some Paclobutrazol treated trees have high Paclobutrazol residue in seeds shows vivipary and deformed seedling after germination of these similar to that of Paclobutrazol treated seeds (Tripathi, 1993). Thus Paclobutrazol appears to cause deformity to Mango seedling similar to ethylene (Tripathi 1993) and malformin (Singh, 1996). Paclobutrazol residue obtained in different parts of fruit was having direct effect of doses and date of application. Which was further coupled with number of years, Paclobutrazol was not applied continue. Paclobutrazol treatment of the present doses appears to be higher, carrying bad effect on fruit like smelling and vivipary seeds and deformation of seedling. However, highest residue obtained in edible parts of fruits are much lower than these acceptable by FAO 0.05 mg/kg.

Therefore, Paclobutrazol can be safely used for Mango production of the present doses 1.0 g/ meter canopy diameter (21 year old) applied in the first year and can be repeated with half does

in second year and cannot applied does in the following years The reduction in growth and increased flowering and fruit appears to be counteraction of gibberellins activities by by Paclobutrazol as well as its Cytokinins at lower concentration , which indicates contracted of gibberellins activity as evidence by their respective bioassay in the present investigation. Therefore suggests that Paclobutrazol contracted gibberellins iduced growth and also as IAA and cytokines at lower concentration.(Data not shown)

Discussion

Paclobutrazol (PBZ), a triazole derivative, has been effectively used to induce and manipulate flowering, fruiting and tree vigour in several perennial fruit crops. However its use in mango is quite common. Soil application of paclobutrazol has been efficacious in promoting flowering and increasing yield in many fruit crops. However, there are some conflicting reports on its impact on fruit quality parameters. Besides reducing gibberellins level, PBZ increases cytokine in contents, root activity and C: N ratio, whereas its influence on nutrient uptake lacks consistency (Data not shown). PBZ has been characterized as an environmentally stable compound in soil and water environments with a half-life of more than a year under both aerobic and anaerobic conditions. However, its residue could not be detected above quantifiable level (0.01 ppm) in soils and fruits when applied in optimized rate. In view of the above, optimized use of the PBZ to derive maximum benefit with least undesirable impact on food and environmental safety aspects is suggested. Fruits collected showed a low percentage of radio activities in the product applied by soil, an average of 1.65% to 4.30% for the pulp and to confirm that the analyses of 14C-PBZ were measured by HPLC. The method was validated using mango fruit samples spiked with paclobutrazol at different concentration levels (0.01 and 0.3µg/mL). Average recoveries (using each concentration six replicates) ranged 89-93%, with relative standard deviations less than 3%, calibration solutions concentration in the range 0.01-2.0µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01µg/ml and 0.03µg/mL respectively. (Table6)

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References

[1] Anonymous. 1984. Technical Data sheet-Paclobutrazol, Plant Growth Regulator for Fruits. ICI. England.

[2] Anonymous, 1986. Technical data Paclobutrazol (plant growth regulator) for fruits. Imperial Chemical industries PLC Plant protection division England.

[3] Anonymous, 1988. Pesticide residue in food-1988. FAO Plant production and protection Paper 93/1, pp. Paclobutrazol, p. 147- 164 J.M.P.R. 1988.

[4] Belmans, K. 1989. Study of growth yield and fruit quality of Sweet Cherry, Cvs. Hedelfinger. After soil application of Paclobutrazol. *Acta Hortic*, 239, 443-446.

[5] Burondkar, M.M. and Gunjate, R.T. 1991. Regulation of shoot growth and flowering in 'Alphonso' mango with paclobutrazol. *Acta Hort*., 291:79-84. [6].

[6] Curtis, R.W. 1985. Root curvatures induced by culture filtrate of *Aspergillus niger*. *Science*., 128: 661-662,

[7] D.R. Jenke, 1998. Development and validation of an ionexclusion chromatographic method for the quantitation of organic acids in complex pharmaceutical products, *Instrumentation Science and Technology*, 36 (4), 179-186.

[8] F.L. Vaz, M.L.B. Milfont, A.M. Souto-maior, E.R. Gouvia, 2007. Determination of concentration of paclobutrazol by high performance liquid chromatography and spectroscopy, *Química Nova*, 30 (2), 281- 283.

[9] G.A. Shabir, 2003. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization, Journal of Chromatography A, 987 (1-2), 57-66.

[10] G.S. Nunis, M.L. Riberio, 1999. Pesticides: Use, Law and Control, *Ecotoxicology and Environment*, 9, 31- 44.

[11] Hasdiseve, E, C. and P. Tongupai 1986. Effects of paclobutrazol on vegetative growth, flowering,

and fruiting of mango •eNam Dok Mai Twai No.4•f. Report at the Kasetsart University Annual Conference, Kasetsart University, February 1986.(in Thai)

[12] Hunter, D. M. and J. T. A. Proctor 1990. Paclobutrazol bioassay using the axillary growth of a grape shoot. *Hort. Science* , 25: 309-310.

[13] Iyer, C.P.A. and Kurian, R.M. 2002. Strategies for High Density Planting of Horticultural Crops. In. Hi-Tech Horticulture. Chadha K.L, Choudhary M.L. and Prasad K.V (Eds.), Horticultural Society of India, New Delhi, pp. 66-78

[14] J.M. Greens, 1996. A practical guide to analytical method validation, *Analytical Chemistry*, 68 (9), A305- A309.

[15] Jaumien, F, M. Winktorowal CZ and B. Osinska, 1986. Vegetative growth control and fruiting of young pear trees treated with CCC, SADH, PP333 (paclobutrazol), and a mixture of these compounds with CEPA. *Acta Horticulturae* 179: 221-225.

[16] Kulkarni, V.J. 1988. Chemical control of tree vigour and the promotion of flowering and fruiting in mango using paclobutrazol. *J. Hortl. Sci.*, 63: 557-66

[17] Kurian, R. M. and Iyer, C. P. A. 1993b. Chemical regulation of tree size in mango cv. Alphonso. II. Effects of growth retardants on flowering and fruit set. *J. Hortl. Sci.*, 68:355-60.

[18] Kurian, R.M. and Iyer, C.P.A. 1993a. Chemical regulation of tree size in mango cv. Alphonso. I. Effects of growth retardants on vegetative growth and tree vigour. *J. Hortl. Sci.*, 68:349-54.

[19] Kurian, R.M. and Iyer, C.P.A. 1993c. Chemical regulation of tree size in mango cv. Alphonso. III. Effects of growth retardants on yield and quality of fruits *J. Hortl. Sci.*, 68:361-64.

[20] Kurian, R.M., Reddy Y.T.N., Sonkar, R.K. and Reddy V.V.P. 2001. Effect of paclobutrazol on source-sink relationship in mango (Mangifera indica L). *J. Applied Hort.*, 3:88-90

[21] Kurian, R.M., Reddy, V.V.P. and Reddy, Y.T.N. 1996. Growth, yield, fruit quality and leaf nutrient status of thirteen-year-old 'Alphonso' mango on eight rootstocks. *J. Hortl. Sci.*, 71:181-86

[22] L.C. Luchini, 1987. Adsorption-Dessortion of Herbicides Paraquat, Diuron and 2,4- D in six Brazilian soils, master diss., Superior School of Agriculture "Luiz de Queiroz", Piracicaba, SP.

[23] L.M.G. SILVA, A.R. SÃO JOSÉ, 2001. Dosage and Methods of Application of Paclobutrazol on Tommy Atkins Hoses, Magistra, 13: 2-3.

[24] M. LANÇAS, 2004. Validation of chromatographic methods of analysis (São Carlos, Ed. RiMa,).

[25] M. Ribani, C.B.G. Bottoli, C. H. Collins, I.C.S.F. Jardim, L.F.C. Melo, 2004. Validation in chromatographic methods and eletroforetics, Química Nova, 27 (5), 771-780.

[26] Mcdaniel, G. L. 1983. Growth retardation activity of paclobutrazol on chrysanthemum. *Hort.Science*, 18: 199-200.

[27] Mauk, C.S.; Unrath, C.R. and Blankenship, S.M.1990. Development of strong cation exchange method for purification and HPLC assay of gibberellins- sterol inhibition inplant tissue. *J Chromatographic Sci.*, 28: 621-623.

[28] P. Tongumpai, N. Hongsbhanich, C.H. Voon, Cultar, 1989. Flowering regulation of mango in Thailand, *Acta Horticulture*, 239, 375-378.

[29] R. A. Creager, 1986. A miniature pressure injector for deciduous woody seedlings and branches. In: Bioassays and Other Special Techniques for Plant Hormones and Plant Growth Regulators.(YOPP, J. H., L. H. AUNG and G . L. STEFFENS eds.) *Plant Growth Reg. Soc. of Amer. Pub.* USA. 181-182.

[30] Reed, A. N. 1988. Quantitation of triazol and pyrimidine plant growth retardants. *J. Chromatogr*. 438: 393-400.

[31] Reynolds, A.G.; Cottrell, A.C.; Wardle, D.A. and Guaunce, A.P. 1991. NAA and Paclobutrazol control grapevine suckers, vine performance and fruit tissue culture.Hort. Sci., 26: 1286-1287

[32] Reynolds, A.G.; wardle, D.A.; Cottrell, A.C. and Gaunce, A. P. 1992. Advancement of Riesling' fruit maturity by Paclobutrazol induced reduction of lateral shoot growth. *J. Amer. Soci. Horti. Sci.*, 117:430-435

[33] Steffens, G. L. and S. Y. Wang 1986. Biochemical and physiological alteration in apple trees caused by a gibberellin biosynthesis inhibitor, paclobutrazol. Acta Horticulturae 179: 433-442.

[34] Sterrett, J. P. 1985 Paclobutrazol- a promising growth inhibitor injection into woody plants. *J. Amer. Soc. Hort.* Sci.110: 4-8.

[35] Swietlik, D. and S. S. Miller, 1985. The effect of paclobutrazol on mineral nutrition of apple seedlings. *J. Plant Nutr*. 8: 396-328.

[36] Tripathi, P.C. and Ram, S. 1994. Induction of malformation like symptoms in mango by Ethephon and Paclobutrazol. *Scientia Hort.*

[37] T J. Tworkoshki, 1985. Response of trees to injection of flurprimidol and Paclobutrazol. Proceedings 39th Annual Meeting of the Northeastern Weed Science Society pp. 215.

[38] Tongumpai, P, K. Jutamanee and S. Subhadars-hanbandhu, 1991. Effect of paclobutrazol on flowering of mango cv. Khiew Sawoey. *Acta Horticulturae* 291: 67-70.

[39] Tymoszuk, S, 1986. Growth control of apple trees with cultar and alar. *Acta Horticulturae* , 179:195-220.

[40] Wiland, W. F and R. L. Wample, 1985. Effect of paclobutrazol on growth, photosynthesis and carbohydrate content of Delicious apple. *Scientia Horti*. 26:131-149.

[41] WILLIAMS, M. W., E. A. CURRY and G. M. GREENE, 1986. Chemical control of vegetative growth of pome and stone fruit trees with GA biosynthesis inhibitors. *Acta Horticulturae*, 179: 453-458.

[42] Wood, B. W. 1986. Influence of paclobutrazol (PP333), flurprimidol (EL-500)and ortho XE-1019 (Chevron)growth retardants on growth and selected chemical and yield characteristics of Carya illinoensis . *Acta Horticulturae*, 179: 287-288.

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