RESEARCH HIGHLIGHTS
2006

UPASI Tea Research Foundation
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Cover photograph: Predatory thrips feeding on red spider mite

CAVEAT

This booklet is highlight of work and investigations in 2006. These should not be regarded as recommendations unless so stated or until a separate recommendatory note on the specific aspect is issued in the Handbook of Tea Culture.

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INTRODUCTION

Plant breeding is probably the most important area of research which can bring in benefits to the future tea replanting programme in south India. Efforts are needed to develop planting materials which are not only high yielders but also of superior quality and suitability to shear or machine harvesting. The Institute is evaluating certain high quality clones from Darjeeling, for planting in the Nilgiris. Four accessions collected from certain estates of south India were found promising, based on the mean cluster values and cluster distance. A selection from High Range, selected a few years back has been found to be of high quality and worth releasing for planting in south India. Similarly, a selection from the biclonal seed stocks and evaluated in Karnataka is a high yielder. It has now been proved that "approach grafting" will be a useful technique for multiplying scarce or rare planting materials.

Certain estates had always found it difficult to establish *Grevillea robusta* due to various reasons. Problems of initial establishment can be solved to a certain extent by raising the silver oak plants in jumbo poly bags. We also found that an indigenous tree species, *Melia dubia* performing well under experimental conditions, in the field. We plan to test this species in a couple of estates which had reported difficulties in establishing silver oak plants.

The blister blight pathogen *Exobasidium vexans* is a hardy fungus which thrives even during off season on tea plants near the ravines and on shoots below the canopy. The lesions sometimes appear on the upper surface of leaves during the dry months. The spores from such lesions are big and thick walled but fail to germinate and do not produce symptoms of disease. It appears that these thick walled spores may have a period of dormancy or require specific environmental conditions to germinate and infect leaf tissue. A review of the susceptibility of the tea clones revealed that two clones are highly resistant, four resistant and seven tolerant to the pathogen. All these resistant and tolerant clones had less than 15% disease severity. However, there had hardly been any attempt to understand the biochemical basis of resistance or susceptibility of the tea plant to *E. vexans*. Treatment of shoots with salicylic acid resulted in the appearance of new protein bands in the resistant clones and these proteins may play a significant role in the response of plants to the blister blight pathogen. Another important work relates to the use of RAPD primers for identifying markers associated with resistance of tea clones to the blister blight pathogen. Six of these markers were sequenced and converted into SCAR markers.

Earthworms play a significant role in the fertility the soil and a survey of the earthworms of Nilgiri tea soils showed the presence of eight species of which *Amynthas corticis, Dravida parva* and *Pontoscolex corethrurus* were more common. Basic studies in the laboratory revealed that the tea soils of south India are highly responsive to potassium fertilizer application. Another lab study showed that net N mineralization of organic manures remained high for about 100 days after application.

In Entomology division the thrust areas of research were semiochemicals and microbial control in addition to the work on pesticide residues. Research on the use of kairomones from *Montanoa* for shot hole borer control and the female sex pheromones of tea mosquito was continued. Attempts were made for the mass multiplication of two major predators of red spider mites. Certain new chemicals were also evaluated in the field for the control of this mite. Surveys were conducted to study the level of pesticide residues in south Indian teas. It is worth mentioning that none of these tea samples were positive for dicofol, DDT, endosulfan, hexaconazole, fenpropathrin, fenvalerate, propargite and quinalphos. The content of lead was extremely low and below the PFA Act limit. Teas from Karnataka were analysed for one year and were found to be absolutely free from pesticide residues through out the year. Studies revealed that repeated application of hexaconazole at 7 days interval resulted in a higher level of residues at 1.22 ppm on the 10^{th} day after application. The content of chromium in black tea was influenced by the sharpening of CTC rollers.

Microbes present in the tea machinery adversely affects the quality of black tea. Sanitrol, a mill sanitizer was effective in reducing the microbial load in tea machinery. The pathology division was also successful in developing a kit for the qualitative determination of microbial load in the tea machinery. This user friendly kit is now available from the TRI. A consortium of *Azospirillum* strains and another of phosphobacteria suitable for application in south Indian tea fields have been commercialized.

Withering is a major step in tea manufacture but there are no definite markers to fix the optimum withering time. Scientists at the Tea Technology division are evaluating a few bio chemical parameters and proline appeared to be a promising biochemical marker for this purpose. Tea catechins are well known for their scavenging properties against free radicals. Galocatechin gallate offered 95% protection of DNA, followed by epigallocatechin. It is also interesting to note that protection afforded by black tea was the highest followed by green tea and oolong tea!

The Regional Centres of TRF undertook more than 1400 visits to the tea gardens for experimental and advisory work. They also conducted various training programmes for the executives as well the estate staff. A large number of samples of tea and agro chemicals were analysed, mostly at the laboratories at Coonoor and Vandiperiyar. The monthly advisory circulars from the Regional Centres were useful to the planters in carrying the regular cultivation practices, more precisely. Area Scientific Conferences were held in Regional Centres. Several of our scientists and Research Fellows participated in the XVII Plantation Crops Symposium and presented papers. A total of 12 papers were presented by the TRI scientists at PLACROSYM. The TRF played an active role in the conduct of the Golden Leaf India Awards 2006, held in Dubai. The tea samples meant for the competition were collected coded and analysed for pesticide residues and heavy metal by the scientists of the Foundation.

UPASI TRF, Valparai
1st April, 2007

(N.Muraleedharan)
DIRECTOR

1. BOTANY

1.1. Controlled hybridization

F1 progenies derived from 36 different combinations, hybridized during 2004 were planted in the field for evaluation of combining ability and heterosis percentage. During the year 30 different crosses were made and the seeds were germinated in the nursery.

1.2. Mutation breeding

Eight more popular clones and three biclonal seed stocks were treated with mutagen and planted in the field to study their variation. Based on the LD-50 values fixed after the previous experiments, three biclonal seed stocks were irradiated with gama source ranging from 8.5 to 20.5 K Gy. As a result of mutation twin branches were noticed in the biclonal seedlings.

1.3. Clonal selection

Genetic diversity and character evaluation were studied in 34 new accessions collected from different tea growing regions of south India. Clustering was done on the basis of preliminary data recorded on 11 different characters. The accessions were grouped into 13 different clusters. Based on the mean cluster values and cluster distance, four accessions were found promising. These accessions were multiplied to study their quality and yield potential in multi locational trials.

1.4. Approach grafting technique for rapid multiplication of TRF-1

TRF-1 plants raised through approach grafting on the root stock clone ATK-1 were planted in the field for field evaluation. Survival of these composite plants was 100 per cent. Graft union at the graft interface was found to be firm after centering the plants above the approach grafted region.

1.5.Germplasm collection, conservation, documentation and registration

A total of 48 accessions collected from the old seedling populations from different tea growing regions of south India were included in the germplasm bank. During the year five accessions maintained in the germplasm bank were allotted National identity numbers by the NBPGR.

1.6. Evaluation of Darjeeling clones in the Nilgiris

Certain popular Darjeeling clones *viz*., TK-78, T-253, RR-144, AV-2, CP-1 and P-312 were multiplied through approach grafting technique using the root stock clones UPASI-2 and UPASI-9. Graft success of these combinations ranged from 80 to 100 per cent. These grafted plants were planted in the field in the Nilgiris to evaluate their suitability for orthodox type of manufacture in the high elevation regions and also for their yield potential.

1.7. Evaluation of certain selections from north Indian estates

Eight selections from north Indian estates were evaluated for their quality and yield potential in the Anamallais. Establishment and initial growth of all the accessions were more

or less comparable to the standard clone UPASI-9. Biochemical evaluation of these accessions revealed that among the eight, four selections have good quality characters.

1.8. Evaluation of alternate shade tree species for shade

Among the six native tree species which were evaluated as alternate shade trees to *Grevillea robusta*, annual growth rate of *Melia dubia* was the highest followed by *Toona ciliata.* Growth of the other rain forest species *Dimocarpus longan, Ormosia travancorica, Filicium decipiens* and *Trichelia connaroides* was lower than *Grevillea robusta*.Observations on the other characters are being continued.

1.9. Raising Grevillea robusta in jumbo poly bags for better establishment.

Grevillea robusta plants were raised in jumbo poly bags of 45 X 15 cm to raise them to a height of more than 120 cm by extending their nursery life for 18 months. In the nursery these plants attained a height of 130 cm with a stem girth of 3.2 cm at 10 cm height from the ground. When these plants were planted in the field, they were taller than the tea canopy and could easily establish without damage from accidental drift of herbicides.

2. PLANT PHYSIOLOGY

2.1. Biochemical basis of resistance/ susceptibility of tea clones

To understand the biochemical basis of resistance/susceptibility character, clones known for resistance/susceptibility against blister blight disease (SA-6, SMP-1, UPASI-15, UPASI-10, UPASI-3, TES-34, UPASI-7 and UPASI-4) were selected for leaf protein analysis using SDS- PAGE. Shoots of each clone were treated with 5mM salicylic acid (as elicitor) and their leaf protein profiles were compared with untreated shoots of respective clones. New protein bands (30 k Da and 32 k Da) appeared in the resistant clones due to salicylic acid treatment when compared to untreated control. Several other protein bands also showed higher activity in salicylic acid treated samples compared to untreated control samples. These proteins may play roles in determining resistance/susceptibility of tea clones to blister blight.

2.2. Molecular characterization of germplasm

Molecular characterization of Indian tea germplasm (including UPASI collections) has been undertaken as a networking project sponsored by DBT, New Delhi. Under this program, UPASI has completed AFLP fingerprinting of 938 tea accessions using two primer combinations alloted to UPASI.

2.3. Protection of DNA by tea catechins from damage induced by free radicals

Tea catechins are powerful antioxidants and have the properties to scavenge free radicals. Protective role of the individual catechins and tea liquors (black tea, green tea and oolong tea) in protecting plasmid DNA (pBR 322) from free radical induced damage was studied. The results showed that individual catechin molecules have high efficiency in protecting DNA from free radical induced

damage. The gallated forms of catechins had higher protection capacity than their epicatechin forms. About 50% of DNA was degraded by hydroxyl radical in the absence of catechins. High protection of DNA was found in gallocatechin gallate (95%) at 0.8mM followed by epigallo catechin at 1mM concentration. The lowest level of protection (61%) was found in epigallo catechin gallate, followed by (+) catechins at 0.4 mM concentration. DNA protection was high due to gallated catechin than by epicatechins. Among the different made tea samples, black tea had the highest DNA protection capacity followed by green tea and oolong tea.

2.4. Hairy root transformation studies

Tea leaves were transformed with *Agrobacterium rhizogenes* (MTCC 532) under optimum conditions. Murashige and Skoog medium supplemented with 30g/l maltose and 5ppm IAA was found suitable for hairy root culture and accumulation of phenolic components. Confirmatory studies on transformation were carried out by PCR analysis using *rol* C gene primer. Amplification of the specific gene was noted in the transformant at 540bp. HPLC analysis confirmed higher levels of catechin and their fractions in transformed roots.

2. 5. RAPD analysis of UPASI clones

DNA from 26 UPASI released clones was analysed using 10 RAPD primers. A total of 1348 marker bands were produced by these primers. Jaccards similarity coefficient values showed a wide range of variation (0.162 to 0.684). The highest similarity was noticed between UPASI-21 and UPASI-22 and lowest between UPASI-16 and UPASI-9. An UPGMA based dendrogram showed five clusters with minimum of 45% similarity comprising 19 out of the 26 clones tested. Remaining seven clusters did not form any cluster. It was possible to classify these 19 clones as Assam, China, Cambod or intermediate group while the remaining seven clones did not show any clear affinity to any of these groups.

2.6. Molecular markers for blister blight resistance / susceptibility

Data generated from eighty RAPD primers were analysed and few marker bands were identified as markers for use associated with blister blight resistance/susceptibility. These selected marker bands were further validated using 31 clones. Six of these markers were sequenced and converted into SCAR markers. Work on PCR conditions for these six SCAR markers to obtain single band in SMP - 1, SA - 6 and TES - 34 and further validation of SCAR markers are in progress.

2.7. Identification of clones through molecular technique

A technique was developed to indentify tea clones using RAPD primers. DNA from 31 tea clones were analysed with 11 primers. It was found that a minimum of three primers are required to identify all these 31 clones.

3. CHEMISTRY

3.1. Zinc toxicity

Distribution and accumulation of soil applied zinc were studied in potted plants. There were nine treatments including an

untreated control, where no zinc was applied. Zinc acetate was added to soils to obtain 8 treatments having 10,25,50,100,500,750,1000 and 2000 ppm of zinc in soil. The plants in the soil containing 2000 ppm zinc died within 15 days while the plants grown on soil containing 1000 ppm zinc died within 36 days after imposing treatments. The plants raised under 750 ppm zinc showed toxicity symptoms such as leaf browning and scorching. The toxic levels of zinc appeared to be 660 ppm in root, 170 ppm in stem and 160 ppm in leaf.

3.2. Influence of heavy metals on urease activity

An attempt was made to analyse the effect of heavy metals like Ni, Cd, Hg and Pb for inhibiting urease activity, which is responsible for the conversion of applied urea into plant available forms like ammonium and nitrate. The soil samples were artificially contaminated with 25µ mole solutions of the above mentioned metals. It was found that inhibition by Hg was the highest followed by Cd, Pb and Ni. Since maximum inhibition was found to be in the range of 60-70%, care should be taken to ensure that these metals are absent in organic manure and mined products applied in tea fields.

3.3. N mineralisation of organic manure Vs soil pH

An incubation experiment was conducted to study net nitrogen mineralisation of organic manure and tea leaf litter against various pH in the soils of Anamallais. The results indicated that net mineralisation of organic manure and the leaf litter was highest on 52nd day when the soil pH was 4.5. Net nitrogen mineralisation remained at a reasonably higher level up to 100 days after application.

3.4. Potassium releasing character of tea soils

Soil samples were collected to study the exchangeable potassium releasing characteristics of tea soils of 24 different agro climatic zones. It was found that the amount of potassium released due to repeated extractions decreased gradually and reached a constant value at the seventh extraction in the case of all zones of Anamallais, Munnar and Coonoor (Nilgiris) regions. In the other regions a constant value was attained at 8th or 9th extraction. The Cobb-Douglas response equation worked out between cumulative K and number of extractions indicated that all zones of south India are highly responsive to potassium fertiliser application.

3.5. â–D glucosidase and â-galactosidase activities in tea

Tea shoots comprising three leaves and a bud were harvested from 20 different cultivars planted in a single area of UPASI Tea Research Farm and analysed for â-D-glucosidase and â-galactosidase activities which regulate carbohydrate metabolism. Among the cultivars studied a range of variability of 0.63 to 5.45 units of â-galactosidase activity and 0.06 to 1.91 units of â-D-glucosidase activity were observed. This result suggested the influence of genotype on these enzymes. The activity was higher in actively growing tea tissues (bud) and lowest in the bark of tea plants. The insignificant correlation coefficient obtained for these two enzymes with productivity suggests

that they could not be used as a biochemical marker to predict the yield potential of tea cultivars

3.6. Concentration of iron Vs iron polyphenol complex

A study was undertaken to assess the effect of iron in the soil on amino acids and polyphenols in tea leaves. Fe was added externally to the soil at concentrations ranging from 0 to 5000 mg per kilogram of soil. The amino acid content increased linearly up to 500 mg Fe kg^{-1} soil beyond which there was a sharp decline. The content of polyphenols increased due to addition of 50 mg Fe kg^{-1} soil and then declined significantly.

The plants, which received more than 750 mg Fe kg^{-1}, exhibited significantly lower amount of polyphenols, compared to that of control. This observation confirmed the department's earlier findings that higher concentration of iron could result in the localized formation of iron polyphenol complex.

3.7. Manganese toxicity

A pot experiment was carried out in the clone UPASI-9 to establish the symptoms of manganese toxicity. There were seven treatments including untreated control. Mn was added externally to make the soil to contain 0, 100, 500, 750, 1000, 3000, 5000 and 7000 mg Mn^{2+} per kg of soil. The toxicity symptoms appeared on 4^{th} day in the treatment having 7000 ppm Mn, on 7^{th} day with 5000 ppm Mn and on 10^{th} day at 3000 ppm Mn. Irregular black spots all over the leaf surface right from the petiole up to the leaf tip between the marginal veins were noticed. Manganese toxicity resulted in the death of plants on 13^{th} day when manganese in the soil was 7000 ppm on 18^{th} day at 5000 ppm Mn and on 23^{rd} day at 3000 ppm Mn. Further, manganese influenced certain enzymes like catalase and biochemical parameters. The amino acid content increased up to the concentration of Mn at 500 mg kg^{-1} beyond which it decreased whereas catalase activity increased till 1000 mg Mn kg^{-1} and then decreased. The contents of polyphenols and catechins increased up to the concentration of Mn at 1000 mg kg^{-1} and there was a sharp decline thereafter.

3.8. Magnesium adsorption capacity of tea soils

Magnesium adsorption capacity of tea soils of three different agro climatic regions viz., Anamallais, Munnar and Vandiperiyar was determined and fitted in Freundlich, Langmuir and Dubinin-Radushkevitch adsorption isotherms. At any point of time, the equilibrium concentration of Mg was the highest for Munnar soils which could be due to the higher soil organic matter status. The higher clay content in Vandiperiyar soils favoured the higher magnesium adsorption. Anamallais soils showed best fit with Freudlich adsorption isotherm (100%) followed by the soils of Vandiperiyar (99.9%) and Munnar (99.8%). In all three regions, the adsorption model developed with Dubinin-Radushkevitch adsorption isotherm yielded poor fit rendering it unsuitable for predicting magnesium adsorption capacity of tea soils. The binding energy of magnesium was highest in Vandiperiyar region (0.00279 mg of Mg kg^{-1})

while soils of Anamallais had higher adsorption maxima. From Dubinin-Radushkevitch adsorption isotherm the mean energy of adsorption was calculated which was lesser for the soils of Vandiperiyar compared to the soils of Munnar and Anamallais. The adsorption of magnesium progressively increased when the concentration of added magnesium increased. About 46 to 52% of added magnesium was absorbed into the soil within 24 hours.

3.9. Aluminium toxicity

A pot culture experiment was carried out in the clone UPASI – 9 to establish the symptoms of aluminium toxicity. Al was added externally to the soil to contain 0, 100, 500, 750, 1000, 2000 and 3000 mg Al kg^{-1} soil. The toxicity symptoms appeared on 5th day in the treatment having 3000 ppm Al, on 7th day with 2000 ppm Al and on 10th day at 1000 ppm Al. The leaf colour changed to pale yellow. Total bleaching of chlorophyll pigments was seen at initial stages and browning all over the leaf surface especially close to the leaf margin and leaf tip was noticed at the end. Aluminium toxicity resulted in the death of plants on 7th day when Al was 3000 ppm in the soil, on 9th day at 2000 ppm Al and on 11th day at 1000 ppm Al.

4. ENTOMOLOGY

4.1. Work on Entomopathogens

The entomopathogenic bacterium, *Bacillus cereus* was made into a liquid formulation and evaluated against red spider mite in the lab and field. Pathogencity bioassays were conducted with culture filtrate @ 1: 25 (spore load 13 x 10^{13}). All the life stages were highly susceptible to the bacterium and cent percent mortality was observed on the 3rd day after application. Significant reduction in red spider mite population was noticed in the field. The impact of *B. cereus* on the natural enemies like *Oligota pygmaea* (Staphylinidae) and *Stethorus gilvifrons* (Coccinellidae) was also evaluated in the lab.

The entomopathogen isolated from thrips has been identified as *Aspergillus flavus*. Pure stock culture of fungus, *Aspergillus flavus* was maintained in SDA slants and the pathogen was mass multiplied in petri dishes (9 cm diameter). Seven days old culture was used in all the experiments. The fully sporulated fungal mat was separated, washed in water, filtered and used at specific concentrations (10^6 - 10^9 spores per ml) to evaluate against thrips. Based on the laboratory results, a micro-plot field study was also conducted using a wettable powder formulation @2000g/ha with encouraging results.

Pathogenicity of *Bacillus circulans* also was tested against nettle grubs and other leaf feeding caterpillars with encouraging results.

4.2. Studies on the attractant chemicals from *Montanoa bipinnatifida*

Chemicals released from the partially dried cut stems of *Montanoa* were captured and identified in Gas Chromatograph coupled with Mass Spectrum. Study revealed the presence of seven important compounds belonging to monoterpene, sesquiterpene and cyclodiene groups. A particular mixture of these compounds attracted sizable number of shot hole borer beetles in the field. Among the traps

tested funnel type was found most suitable to trap shot hole borer. Optimization of trap height and number of traps /ha were also done by large scale field trials.

4.3. Sex pheromones of tea mosquito

The composition of the compounds involved in the attraction of sex pheromones of tea mosquito was determined. Based on the field studies two blends of these compounds were fine-tuned and found effective in attracting the male tea mosquito. Expeiments on synthesis, blending and evaluation of suitable traps were standardized.

4.4. Studies on semiochemicals

Volatiles emitted from the red spider mite infected tea leaves were identified using the GC-MS. Eight important compounds belonging to terpenoid and ester groups were identified. Bioassays were conducted using the 'Y' tube olfactometer using total extract of these volatiles. The results indicated that these volatiles attracted predators such as *Oligota pygmaea* and *Stethorus gilvifrons* feeding on red spider mite.

4.5. Efficiency of important predators of RSM

Comparative predatory efficiency of the larval instars and adults of the common predators such as *Oligota pygmaea*, *Stethorus gilvifrons*, *Mallada boninensis* and *Chrysopa madestes* were carried out in the lab. Among the four species, *O. pygmaea* and *S. gilvifrons* consumed significantly more number of mites than *C. medestes* and *M. boninensis.* Occurrence of these predators in large numbers during the peak periods of red spider mite has been confirmed through field studies.

4.6. Mass multiplication and field release of important predators of RSM

An attempt has been made to mass multiply the common predators of red spider mite such as *Oligota pygmaea*, *Stethorus gilvifrons* under lab conditions both on natural and semi-synthetic diets. 200 adults of *S. gilvifrons* were released in the mite infested tea bushes (covered with net) to evaluate their predatory potential

4.7. Biochemical changes in the tea leaves due to thrips infestation

Due to thrips infestation, the chlorophyll content and photosynthetic rate of tea leaves were adversely affected and the tea, manufactured from thrips infested tea shoots, had lower amount of theaflavin, thearubigin, total liquor colour, highly polymerised substances and water extract.

4.8. New acaricides for RSM and TM control

Certain new chemicals were evaluated against red spider mites at Valparai and Vandiperiyar. Clofentezine (Apollo) 50 % SC@ 500 ml, Milbemectin (Milbeknock)1 % E C @ 340 ml, were found effective against red spider mites. Two new insecticides Thiacloprid (Calypso 240 SC) and Clothianidin (Dantop 50 % WDG) were found effective against tea mosquito.

4.9. Pesticide residues

4.9.1. Survey on residues and heavy metals in south Indian tea

A survey was conducted to find out the residues of commonly used pesticides and heavy

metals in south Indian tea. About fifty samples were analysed for residues of ethion, dicofol, endosulfan, DDT, quinalphos, hexaconazole, fenpropathrin, fenvalerate and propargite and the heavy metal, lead (Pb). Among the samples analysed, only 8 % of the samples were positive for ethion but well below the EU MRL of 3 ppm. None of the samples were positive for quinalphos, dicofol, DDT, endosulfan, hexaconazole, fenpropathrin, fenvalerate and propargite. The level of lead in all the black tea samples was below the PFA Act limit of 10 ppm.

4.9.2. Survey on pesticide residues in Karnataka

A survey was conducted for a period of one year to find out the residues of commonly used pesticides in Karnataka. Every month, 11 black tea samples were collected from 11 tea factories and a total of 130 black tea samples were analysed for ethion, dicofol, endosulfan, DDT, quinalphos, hexaconazole, fenpropathrin, fenvalerate and propargite residues. The results of analysis revealed that black tea samples collected from Karnataka were free from the pesticide residues.

4.9.3. Residues of new chemicals

Analytical methodology was developed for profenofos, propiconazole and cypermethrin in tea. Field trials were conducted in wet and dry seasons to determine the residues of profenofos, fenazaquin, lamdacyhalothrin and cypermethrin in tea. Residues of all these chemicals exponentially dissipated and followed first order dissipation kinetics. Based on the experimental data, safe harvest intervals were determined for use of these chemicals in tea.

4.9.4. Chromium in black tea

To know the influence of sharpening of rollers on Cr content in tea, a study was conducted in the mini CTC manufacturing unit. For this purpose rollers were installed after sharpening and black tea was manufactured on 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day after sharpening. The drier mouth tea samples were subjected to microwave digestion following AOAC method and analysed in AAS. The results indicated that the main source of Cr contamination was the sharpened rollers. To compare Cr level, few black tea samples, collected from estates were analysed. It was found that in CTC black tea, level of Cr content varied from 3-21.2 ppm, though in the majority of samples (90%) Cr was below 10 ppm. In most of the orthodox black tea samples Cr content was only around 1 ppm.

4.9.5. Hexaconazole residues

A study was conducted to determine the residues of hexaconazole, after its repeated application at 200ml per ha in combination with copper oxychloride (210 g) at 7 days interval as per the field practice. A total of five sprays were given on '0', 7th, 14th, 21st, 28th day and the samplings were done at 10 days interval (10th, 20th and 30th day after application). The study revealed that repeated applications of hexaconazole led to a higher level of residues 1.22 ppm on the 10th day.

5. PLANT PATHOLOGY

5.1. Evaluation of two Copper hydroxide formulations (XL 57 DP and XL 77 WP) against blister blight

Two copper hydroxide formulations, 57 DP (Dry Prill) and 77 WP (Wettable

Powder) were evaluated against blister blight. Straight application of DP formulation at 560g/ha was superior to WP formulations sprayed at 700g/ha. The metallic copper content of DP formulation was 57% and that of WP 77% which resulted in the superiority of Dry Prill formulation in bioefficacy.

5.2. Effect of spray volume and fungicide concentration when knapsack sprayer was used in blister blight control

Different spray volumes (150,200, 250,300,350,400,500 litres/ha) were used in knapsack sprayer for blister blight control in tea under plucking. In higher spray volumes (250-500 l/ha), apart from the recommended fungicide dose, higher concentration of fungicides were also included. The bioefficacy was good at recommended fungicide dose at spray volume upto 200 litres and in higher spray volumes the efficacy was inferior. With higher concentration of fungicides, the bioefficacy was good with a spray volume of 250 litres and thereafter it declined with increasing spray volumes. This is due to the dripping loss of spray fluid from the crop canopy when higher spray volumes are employed. So for blister blight control in tea under plucking, when knapsack sprayer is used the ideal spray volume will be 150-200 litres/ha. There is no advantage in increasing concentration of fungicides at higher spray volumes.

5.3. Efficacy of paraffinic oil in blister blight control

Paraffinic oil was tested with copper formulations (Copper Oxychloride - Blitox 50WP) and (Copper hydroxide - XL 57DP) against blister blight. The bioefficacy of XL 57DP @ 300g/ha along with paraffinic oil at 500ml/ha was significantly superior to Blitox 50WP + Paraffinic oil sprayed at the same dosage.

5.4. Efficacy of Sanitrol in reducing microbial load of tea machinery

Sanitrol, a mill sanitizer was tested to find out its efficacy in bringing down the microbial load in tea processing machineries. Sanitrol (4ml/l) was sprayed with a knapsack sprayer after normal washing of machineries (factory practice). Sanitrol spray was effective in reducing the microbial load and its effect lasted for more than 15 hours. while in normal washing, there was a gradual increase in population of microbes.

5.5. Effect of PGPMs in tea nursery

Microbes *Azospirillum, Bacillus, Pseudomonas, Serratia and Trichoderma* with plant growth promoting activity were tested in tea nursery. Application of these microbes in nursery soil enhanced the soil enzyme activity (Phosphatase), and in the plant there was an increase in the activity of PAL and ß 1-3 Glucanase enzymes indicating its ability to trigger systemic resistance. Besides this, the plants treated with PGPMs exhibited good growth compared to untreated ones.

6. TEA TECHNOLOGY

6.1. Identification of biochemical markers for chemical wither

To assess the degree of chemical wither, biochemical constituents such as polyphenols, catechins, amino acids and soluble sugars were estimated in the samples obtained after different hours of withering. Withering up to sixteen hours

had resulted in maximum amount of polyphenols and catechins. The amount of catechins leaching out of the withered leaves was also found to be more in the samples withered for sixteen hours. A decline in the reducing sugar levels was observed as withering progressed up to twenty hours. Flavour assessment during different stages of CTC black tea manufacture was also carried out. As the duration of withering increased there had been an increase in the desirable aroma constituents. Samples withered for sixteen hours had the maximum quality in terms of desirable aroma constituents. A gradual decline in pH was observed in the samples collected from various hours of withering. As a part of the above study, the content of proline was also identified as a marker to assess the chemical wither. The content of proline increased continuously as the duration of withering increased.

6.2. Impact of coarse leaves on quality parameters of CTC black tea

Impact of varying proportions of coarse leaves on the quality constituents of black tea was studied. As the proportion of coarse leaves increased from 25 to 50 % a 24 % decrease in theaflavins and 5 % decrease in water extract were observed. Observations on the optimum fermentation time revealed a 10 minutes difference between the 50 % coarse leaves and 100 % fine leaves treatments. Varying proportions of coarse leaves in the harvest influenced electrical consumption during processing. Observations from the present study indicated a deterioration in quality constituents and increase in the energy consumption as the coarse leaf content in the raw material increased from 25 to 50 %.

6.3. Influence of substrate addition on liquor parameters of CTC black tea

Polyphenol was extracted from green tea and the yield was 16 %. The extracted polyphenol was added to the *cut dhool* at varying proportions on made tea basis. The substrate enriched black teas had higher theaflavin and total liquor colour over the untreated control. Polyphenols and radical scavenging activity were higher in moderate treatment as compared to low and high treatments.

6.4. Comparative study on day and night manufacture

Experiment was conducted to compare the difference in quality of teas in day time and night manufactured. Green leaf having a fine leaf standard of around 85 % (comprising three leaf and a bud, two leaf and a bud, one and two leaves and a soft banji and immature shoots) were used for the experiment. Various physical parameters as well as the optimum fermentation time were recorded both in day time as well as night manufacturing. The fermentation time was delayed by ten minutes in the samples taken during manufacture at night. The level of theaflavins and total liquor colour also showed a marked increase in the samples manufactured during night, probably due to the lower temperature which prevailed during night.

6.5. Assay of pectinase in the crop shoots of UPASI tea clones

Pectinase activity in the crop shoots of UPASI released clones was studied. Maximum

activity was observed in the clone UPASI–22 followed by UPASI-3 and UPASI-2. Component level variation in pectinase activity was also studied. Third internode had the highest activity followed by second internode, third and second leaves. Unlike other enzymes related to tea quality, pectinase activity increased as the maturity of shoots increased.

6.6. Study on optimum fermentation time in orthodox tea manufacture

As part of the studies to determine the optimum fermentation time in orthodox manufacture for various agroclimatic zones, an experiment to determine OFT was conducted at Mailoor tea factory. The chemical analysis of the fermented dhool was carried out for every 10 minutes of fermentation starting from 60 minutes to 180 minutes. The fermented dhool was dried in miniature drier for every 30 minutes starting from 60 minutes. The samples were analysed for quality parameters in the laboratory. The results indicated that optimum fermentation time was 130 minutes in which maximum theaflavin formation was observed. The study is in progress.

7. ADVISORY SERVICE

During the year, a total of 1435 visits were made by our extension scientists to the member estates in connection with advisory and experimental work. They had conducted 50 training programmes on various aspects of tea husbandry for the benefit of the executives and field staff. Visits were also undertaken to the small growers' tea gardens. More than 3300 soil samples, 427 leaf samples, 830 agro - chemicals and 1186 tea samples were also analysed, by the Regional centres.

Area scientific conferences were organised in Nilgiris, Vandiperiyar, Gudalur and Koppa. Scientists and planters belonging to the respective districts discussed the research findings. More than 36 field experiments were in progress in different centres. Regular advisory circulars were sent by the Advisory Officers to the member estates in their respective areas.

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