Indian Institute of Spices Research

Research Highlights

1996-97

An Institute of Indian Council of Agricultural Research

Published by
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Front cover
Shoot morphogenesis in ginger

Back cover (Top to bottom)
gus gene expression in ginger tissues
A ginger accession with bold rhizomes
Pasteuria penetrans spores on *Radopholus similis*

IISRR H- 10.

Director's Introduction

The year 1996-97 marked spectacular growth in spices export. The export touched1.93 lakh tonnes valued Rs.1017.4 crore upto February 1997, an all time record. Black pepper production inKerala alone went upto 59930 tonnes and productivity to 314 Kg/ha during 1995-96.

The management measures suggested by the Institute against *Phytophthora* foot rot in black pepper have received farmers' acceptance and confidence. Biotechnological research in spices, especially protoplast culture and DNA isolation, is giving desired dividends. *Verticillium chlamydosporium* continued to show promise to be an effective biocontrol agent against *Meloidogyne incognita* and even *Phytophthora capsici*. The 'katte' and rhizome rot tolerant lines in cardamom are being multiplied and evaluated further.

The Division of Crop Improvement and Biotechnology operated 18 research projects; 9 in Genetics and Plant Breeding, 3 in Horticulture and 6 in Biotechnology. Research on Paprika was initiated. The Division of Crop Production and Post-Harvest Technology operated 12 projects; 7 in Agronomy and one in Soil Science and 2 each in Biochemistry and Plant Physiology. Research on 'organic spices' and 'clean spices' was initiated. The Division of Crop Protection operated 13 projects; 4 in Plant Pathology, one each in Entomology and Nematology and 3 in Biological Control and 4 in Crop Protection. The stunted disease was attributed to CMV. Biocontrol research, especially against *Phytophthora* foot rot in black pepper, rhizome rot in cardamom and soft rot in ginger, is extended to farmers' plots. The section of Social Sciences handled 5 projects, 4 in Agricultural Extension and one in Statistics. The 'Kurumulaku Samrakshana Samitis' were assessed for their effectiveness in transferring the techchnologies for the management of foot rot of black pepper. The Kodenchery Panchayat (Calicut District) was adopted for the all-round development. Four short term trainings were organised on various facets of spices' research and development. Quality planting materials of various spice crops were supplied to different developmental agencies and farmers under the Integrated Programme for Development of Spices (IPDS). The total income generated during 1996-97 through sale of planting materials was Rs.12.69 lakh. In addition to the institute's programmes, twelve ad-hoc research schemes, 10 funded from AP Cess funds of ICAR and 2 funded by DBT were also in operation. Four new research schemes were approved for funding by ICAR, New Delhi.

The All India Coordinated Research Project on Spices with 20 centres spread over 16 states functioned as per the targets fixed. The Krishi Vigyan Kendra, Peruvannamuzhi conducted frontline demonstrations and organized several training programmes. During the year, the library subscribed to 15 foreign and 80 Indian journals. The total possessions are 3331 books, 2460 bound journals, 2023 reprints and 431 technical reports.

The Agricultural Research Information System (ARIS) was established during the year. A foundation stone was laid for a modern Hardening Facility. Dr. M.S. Swaminathan, Prof. V.L. Chopra, Dr. T.N. Anantha Krishnan, Dr. K.L. Chadha and Dr. R.N. Pal are a few of the notable dignitaries who paid a visit to the institute during 1996-97.

The budget of the Institute was Rs.90 lakh under Plan and Rs.120 lakh under Non-Plan. Besides, Rs.36.88 lakh under AP Cess Fund Schemes, Rs.36.64 lakh for KVK, Rs.9.84 lakh under IPDS, Rs.6.09 lakh under DBT sponsored schemes were also received. The Institute also operated the budget of AICRP on Spices and distributed Rs.45.45 lakh to different centres. The year also witnessed levying charges for various services rendered by the institute.

The Perspective Plan of the Institute was finalised. The Institute provided technical support to Kerala Planning Board in formulating IX Plan proposals of the Government. It also provided technical support to formulate 'Technology Mission on Black Pepper' of the Government of Kerala.

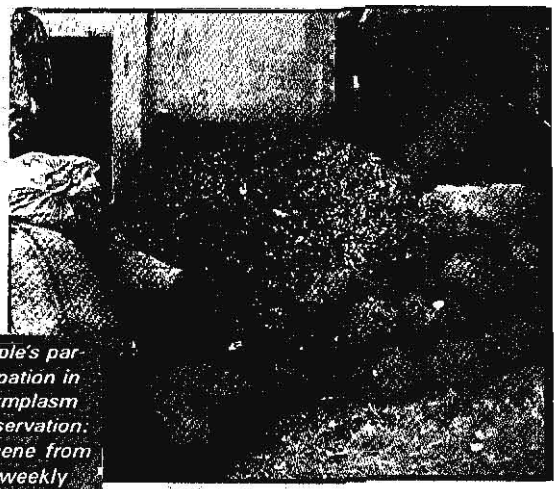
The X Staff Research Council was held during 6-8 May 1996. The mid-term review of the research projects was conducted during November 6-7, 1996. The Study Circle of the Institute met 18 times and approved 15 original research papers. The Post-graduate Committee met 4 times. One scientist was deputed to West Germany and 8 Scientists to research institutes within the country for advanced training. The Institute Management Committee met once. The Institute Joint Council met four times during the year.

The Institute in collaboration with the Indian Society for Spices, Calicut organised the National Seminar on Biotechnology of Spices and Aromatic Plants during April 24-25, 1996. The Institute also organised the first interface between the Spices Exporters Forum and scientists of IISR on 11 October 1996. The meeting of Project Monitoring Committee of DBT on IPM was also organised by the institute during September 18-19, 1996.

Incidence of stunted disease in black pepper and fall in price of cloves and ginger were a few constraints during the year. Standardization of analytical laboratories as per ISO 9002 specifications are to be done on a priority basis. Budget allocated to the Institute during the year was inadequate to meet many essential requirements. The Experimental Farm, Peruvannamuzhi needs protection from wild animals by erecting a compound wall. The CRC, Appangala needs more land for research purpose. Human Resource Development in frontier areas of science, especially genetic engineering, has not received needed attention during the year.

Peter

(K.V. PETER)
DIRECTOR



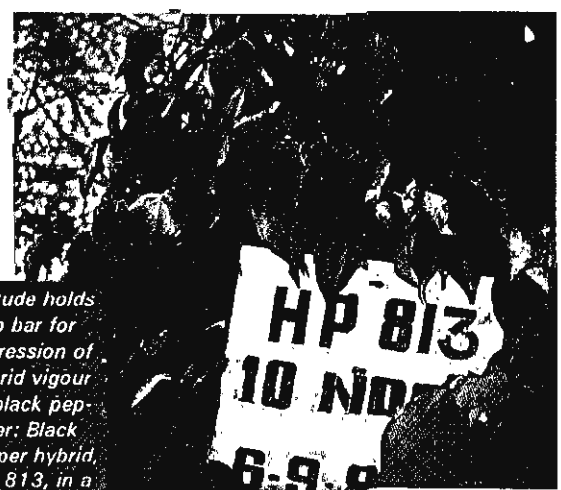
People's participation in germplasm conservation: A scene from a weekly countryside market at Parakode, South Kerala

Division of Crop Improvement & Biotechnology

Crop improvement programmes are oriented towards enlarging the genetic variability in spices through collection and characterisation of germplasm accessions; inter specific hybridisation and through cell culture derived somaclonal variation. Modern biotechnology tools are also employed in pursuing these goals. Development of suitable techniques for vegetative and micropropagation of selected spices is another mandate of the division. Simultaneously, several fundamental studies that give valuable insights into the genetic basis of biological processes are also being carried out in the division.

CONSERVATION OF GENETIC RESOURCES

The institute possesses a treasure of spice germplasm that is enriched every year through collection from different parts of the country. During the year 64 high altitude accessions of *Piper* from Nilgiris, 20 accessions from Valparai and 6 accessions from Mudibe (Chickmagalur) were collected and added to the germplasm repository. The ginger gene bank was enriched with 48 accessions of cultivated types from Vadukkumcherry (Palghat Dist.) and Parakkode (Pathanamthitta Dist.) in Kerala. Two collections, one each from Surat and Sikkim, and one collection from Dehradun were also made. One accession of turmeric, collected from Thatta region (Quilon Dist.), was added to the turmeric germplasm collections. Fourteen accessions of vanilla from Chickmagalur and Dakshnia Kannada were collected besides one from Karuvarakkundu (Nilambur). In tree spices, eight cultivated and five wild nutmeg types, three species of *Syzygium*, one cultivated cinnamon type and five *Cinnamomum* spp. were collected after surveying Anamalai hills, Nilgiris (Tamil Nadu), Nilambur, Koorachundu, Karuvarakkundu (Kerala), Chickmagalur, Uttara and Dakshina Kannada (Karnataka). In paprika, a total of 55 exotic



Altitude holds no bar for expression of hybrid vigour in black pepper: Black pepper hybrid, HP 813, in a garden at Valparai (3500 ft above MSL)

(from USA and Germany), 12 indigenous lines and 126 collections of the Byadagi Dabba type paprika from Dharwad area in Karnataka were collected.

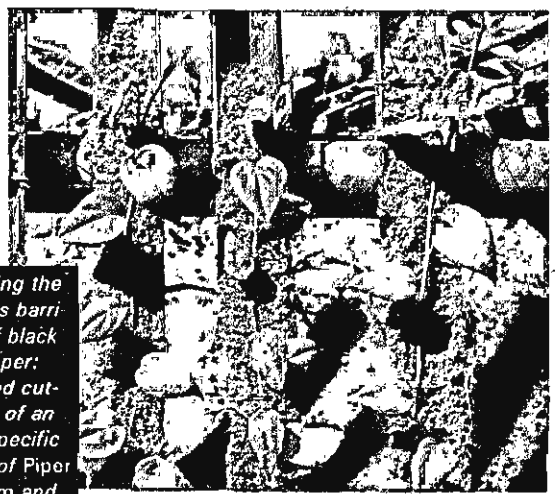
CROP IMPROVEMENT

New varieties in pipeline

Black pepper hybrids HP 34, HP 105, HP 728, HP 759, HP 800 and HP 813 and cultivar 1041 are the promising lines identified for high altitude areas of South India. Among the Wynad accessions of cardamom, vazhukka clones and APG-223 are superior. OP seedlings of Cl_{37} yielded significantly high compared to SKP-21, CCS-1 and Malabar bulk. Two bold rhizome selections of ginger have performed consistently well for the second consecutive year at Peruvannamuzhi (see back cover). In clove progeny trials, B-95 performed better regarding growth characters. Among the *Cinnamomum cassia* collections, three lines with high bark oil were identified.

Interspecific hybridisation

Two interspecific hybrids, one each of *P. nigrum* x *P.attenuatum* and *P. nigrum* x *P. barberi*, have been produced. Characterisation of these hybrids is in progress.



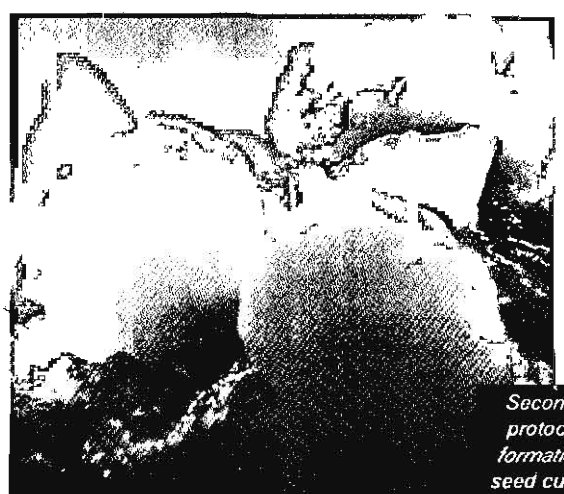
Breaking the species barriers of black pepper: Rooted cuttings of an interspecific hybrid of Piper nigrum *and* P.attenuatum

Cytogenetics and reproductive biology

In ginger, B chromosomes were identified in four accessions. The growth media for the pollen germination and pollen tube growth were standardised. It consists of 15% sucrose, 80 ppm boric acid and 120 ppm calcium nitrate in a moist container maintained at 25°C.

BIOTECHNOLOGY

Protoplasts were isolated from *in vitro* derived leaf tissue and cell suspension cultures in cardamom and turmeric. Callus cultures were induced from seedlings and pericarp explants of paprika. In collaboration with the Madurai Kamaraj University, transient gene expression of *gus* was successfully induced in ginger and cardamom embryogenic cultures using biolistic particle delivery system (see back cover). Kanamycin sensitivity of *P. colubrinum* was assayed and 250 mgl^{-1} of Kanamycin was found to be the level for inhibiting callus growth and differentiation. About 100 seed derived progenies of *Vanilla planifolia* were obtained by seed culture to induce variability in vanilla. Multiple shoots were induced in seedling explants of allspice. Ontogeny of plant regeneration in ovary derived callus cultures of ginger indicated both organogenesis and embryogenesis. In cardamom, 350 somaclones were transferred to soil.



Secondary protocorm formation in seed cultures of Vanilla planifolia

Multiple shoots in a seedling explant of allspice, Pimenta dioica

Developmental Morphology of Ginger and Turmeric

Comparative morphological and anatomical characters of four species each of *Zingiber* and *Curcuma* were studied. Rhizome maceration, comparative leaf anatomy, stomatal pattern, stomatal index and oil cell dimensions were carried out in four *Zingiber* species. Comparisons of root and root tuber anatomy were made in four *Curcuma* species. Histological localization of carbohydrates, starch, lipids, proteins and fibres were done by various staining methods. (See front cover).

Rootstock Studies in Black Pepper

Successful grafts of *P. nigrum* and *P. colubrinum* were obtained through different methods of grafting viz., cleft, saddle, splice, modified splice, tongue and double grafting, the best being double grafting (78%). Mature shoots of *P.colubrinum* were ideal for grafting and two noded scions of black pepper sprouted earlier. None of the above methods succeeded in the case of *P. arboreum*.

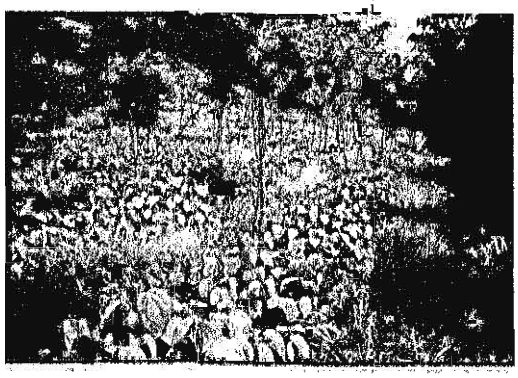
Division of Crop Production & Post Harvest Technology

Crop production programmes are formulated to develop a comprehensive approach to meet the challenges of crop productivity. Development of cropping systems, effective management of inputs, particularly fertilizers and water, use of biofertilizers, drought tolerance and identification of high quality spices are given high priority. Programmes on post harvest technology of spices are also being initiated.

MANAGEMENT OF INPUTS

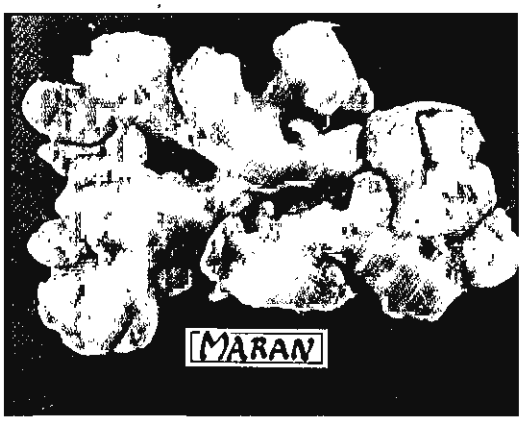
Application of NPK @ 150:60:270 with micronutrients Zn, B and Mo @ 5:2:1 Kg/ha was optimum for pepper varieties Sreekara and Subhakara. Application of Zn, B and Mo @ 5:2:1 Kg ha^{-1} increased the yield of ginger and turmeric also, in the preliminary studies.

Bush Pepper in Field



Planting of bush pepper in the field at a spacing of 2x2 m has realised about one tonne of black pepper per hectare.

Potassium sulphate was better than potassium chloride as a source of K for black pepper. There is no significant difference between different rock phosphates and super phosphate in improving the growth and yield of ginger and turmeric. Drip irrigation @ 7 litres vine^{-1} day^{-1} during October-March increased the yield of black pepper by 67 per cent. Consumptive use of water for adult pepper vine ranged from 2.5-3.0 mm day^{-1} during June-May while this was 3.0-3.5 mm day^{-1} in 16 year old nutmeg trees. Combined application of biofertilizers viz., *Azospirillum*, phosphobacteria and VAM enhanced the growth of black pepper.



Salted Ginger

Salted ginger is prepared using tender bold rhizomes with less fibre. The rhizomes are harvested, cleaned and soaked in sodium chloride and citric acid at a pH below 5 and specific gravity 18 - 20 for about 2 - 3 weeks. The outer skin is removed and preserved in brine. Harvesting at tender stage (150-170 DAP) was found ideal for 'salted ginger' preparation and Maran and Varada are highly promising for this. This is used in preparations of fresh meat and fish in Japan and Middle East.

AGROPHYSIOLOGICAL STUDIES

Subhakara and Panniyur-1 were found excellent among 10 black pepper varieties with respect to their agronomic characters and metabolite contents.

Drought tolerance

Two black pepper accessions, No.1114 and 4057, among 20 identified showed better stress tolerance with respect to stomatal resistance, transpiration rate, cell membrane stability and enzyme activity such as catalase, peroxidase and SOD.

PRODUCTION OF NUCLEUS PLANTING MATERIALS

During the year, about 75,000 black pepper rooted cuttings, 700 black pepper rooted laterals, 20 tonnes of turmeric, 2 tonnes of ginger, 2000 cinnamon seedlings, 1000 clove seedlings, 500 nutmeg grafts, 500 vanilla rooted cuttings, 15,000 cardamom seedlings and 210 Kg of cardamom seed capsules were produced and distributed to various developmental agencies and farmers.

BIOCHEMICAL STUDIES

Maturity studies

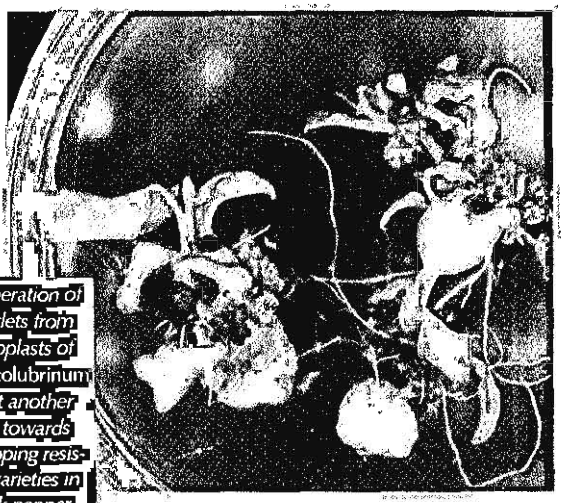
The levels of oil, oleoresin and piperine reach their maximum during 170-180 days after flowering in black pepper and stabilise by 210-230 days. The starch content in the leaf and berries showed a constant increase during berry development. Phenyl alamine ammonia lyase (PAL) had 2.5 fold more activity in the berries compared to leaves and its activity was maximum around 150th day. In ginger, the maximum oil and oleoresin were observed at 120 DAP. Dry recovery in Varada reached stability at 180th day while in Maran it took 210-240 days.

Quality evaluation

Among the 14 ginger germplasm accessions with bold rhizomes, Acc.15 and 71 had 2% oil; Acc.179, 244 and 294 had more than 6% oleoresin and Acc.1717 and 179 had less than 4% crude fibre. Cassia accessions, A_2, D_1 and D_3 recorded the maximum bark oil percentages of 2.8, 2.4 and 2.5, respectively.

Biochemical characterisation of ginger and turmeric

Twenty five ginger and 15 turmeric accessions were screened for their variability using isozyme patterns (acid phosphatase, esterase, catalase, SOD, polyphenol oxidase and peroxidase), protein profiles (SDS-PAGE), major and secondary metabolites. Generally the collections from the same geographical area showed more similarity and clustered together indicating duplications in the collections.



Regeneration of plantlets from protoplasts of Piper colubrinum is yet another step towards developing resistant varieties in black pepper

DIVISION OF CROP PROTECTION

The Division of Crop Protection plays a key role in improving the productivity in spice crops through effective management of pests and diseases. It carries out researches on etiology and epidemiology of major diseases, taxonomy of important pathogens, insect and nematode pests, integrated pest and disease management involving the use of resistant or tolerant varieties, natural pesticides and biological control. Sustainable agriculture by way of non chemical, environment friendly pest and disease control measures is given high priority. Attempts are also made to devlop resistant varieties using modern biotechnological tools.

ETIOLOGY

Electron microscopic studies confirmed the etiology of stunted disease of black pepper as cucumber mosaic virus (CMV). These studies clearly established the presence of CMV in disease affected black pepper leaves. *Cylindrocladium quinquiseptatum* has been confirmed as the causal agent of 'shot holes' of nutmeg. Interaction studies with *M.incognita* and *Pythium aphanidermatum* clearly established the role of *M.incognita* in predisposing ginger plants to soft rot disease. Plants pre-inoculated with *M.incognita* succumbed to infection earlier, compared to plants infected with *Pythium* alone.

PEST & DISEASE RESISTANCE

Screening of germplasm

Among the 50 Kottanadan selections of black pepper, three lines viz., 2571, 2575 and 4255 showed tolerant reaction to *Phytophthora capsici*. In the field trials involving *Phytophthora* tolerant lines in diseased gardens at Sirsi and Peruvannamuzhi, P_{24} stayed healthy. One hundred and sixty eight cardamom clonal accessions were assessed for foliar diseases like leaf blight,

leaf blotch and *Cercospora* leaf spot. Fiftysix black pepper accessions were found susceptible to pollu beetle, *Longitarsus nigripennis*. However, one accession (No.2070) of wild *P. nigrum* was free of pest infestation. Acc.No.176, 233, 291, 569, 606, 614 and 624 were found highly susceptible to root knot nematode, *M.incognita*, while Acc.No.1040 and 1090 are highly susceptible to the burrowing nematode, *Radopholus similis*. Two turmeric (Acc.No.84 and 179) and two ginger accessions (Acc.No.36 and 59) showed high degree of resistance to *M.incognita* (EMI <3; pf<1) in the second round of screening.

Biochemistry of disease resistance

The ratio of total phenol to orthodihydroxy phenols (OD phenols) was high in susceptible varieties compared to that in *Phytophthora* tolerant lines.

Biotechnological approaches

Protocols were standardised for regenerating plantlets from protoplasts isolated from mesophyll cells of *P. colubrinum*. Among the somaclones developed through tissue culture, No.456 has shown tolerant reaction to *P.capsici*. In cardamom, 368 somaclones were screened against mosaic virus and all were found to be susceptible.

Variability in root knot nematodes

Root knot nematode populations collected from different locations and various spice crops show considerable variability. Perineal patterns of *Meloidogyne* spp. help in the preliminary identification of the species involved. Five populations studied during the year showed high variations in their perineal patterns. Differentiating the root knot nematode populations through isoenzyme studies is presently underway.

BIOLOGICAL CONTROL

Diseases

Field trials on biological control of *Phytophthora* foot rot disease of black pepper have shown that *Gliocladium virens* was better than *Trichoderma harzianum* in preventing the root rot. The field trial with VAM and biocontrol agents showed better growth and less mortality (less than 5%) in treated vines. In a large scale demonstration of biocontrol for foot rot in coffee pepper mixed cropping system in Karnataka, foliar yellowing of treated vines decreased from 3.9 to 2.6% and vine death from 20 to 13.5%. *In vitro* studies have shown that potassium phosphonate (Akomin) was compatible with both *T.harzianum* and *G.virens*. *V.chlamydosporium* inhibited the growth of *P.capsici* in dual culture.

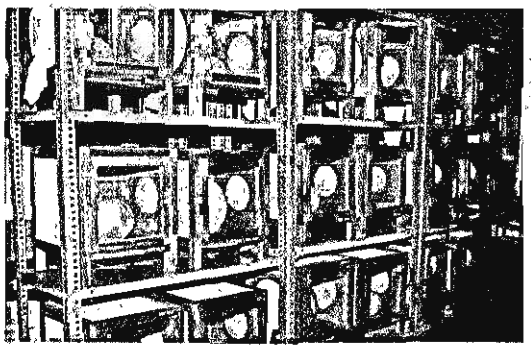
In a black pepper plot with 10 cultivars under integrated disease management of foot rot disease, involving soil application of biocontrol agents and spray and drench with potassium phosphonate, 83.5% plants were healthy and 16.5% showed varying degrees of foliar yellowing ranging from 25% to 37.5%. All P_{24} plants were healthy.

Insect pests

Beauvaria bassiana caused about 20% mortality of the pollu beetle in the laboratory bioassays.

An adult pollu beetle of black pepper infected with the fungal parasite, Beauvaria bassiana

Mass Culturing of Natural Enemies

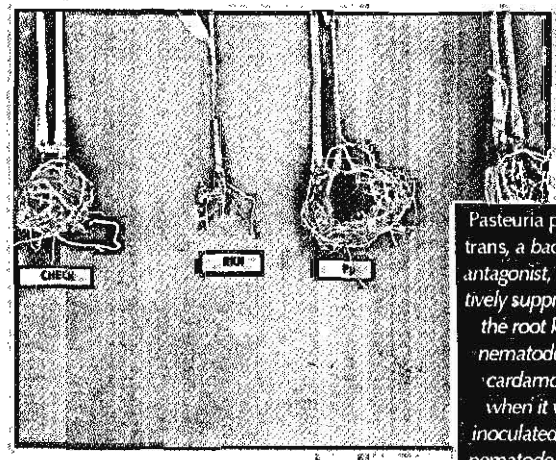


Mass culturing of *Chilocorus circumdatus* and *C.nigrita*, the natural enemies of scale insects infesting black pepper, was standardised. They are reared on the scale insect, *Aonidiella orientalis*, raised on pumpkins in the laboratory.

Bracon sp.and *Apanteles taragamae* (Braconidae) were the most important hymenopterous parasitoids of *Conogethes punctiferalis*, the shoot borer of ginger. Release of the predator, *C. circumdatus* on black pepper vines infested with the scale insects, *Aspidiotus destructor* at Kuppadi (Wynad) reduced the incidence of pest. Neem oil 0.3%, Neemgold 0.3% and fish oil rosin 3% were found safe to *C. circumdatus*, while dimethoate 0.1% and monocrotophos 0.1% are toxic upto 1 and 7 days after treatment, respectively. Evaluation of commercial products of *Bacillus thuringiensis* against shoot borer of ginger and turmeric in the field indicated that four sprays (during July, August, September and October) of Dipel 0.3% was more effective in controlling the pest infestation.

Nematodes

Inoculation of the biocontrol agent, *Pasteuria penetrans*, simultaneously with root knot nematodes or 15 days after nematode inoculation significantly reduced the nematode population and increased the total biomass of cardamom plants.



Pasteuria penetrans, a bacterial antagonist, effectively suppressed the root knot nematodes of cardamom, when it was inoculated after nematode infection

Natural products for pest management

Endosulfan 0.05% spray during July followed by three sprays of Neemazal 0.05% or Neemgold 0.3% during August, September and October were found effective in controlling 'pollu' beetle of black pepper. Neem oil 0.3%, Neemgold 0.6% and fish oil rosin 3% were effective against the scale insects *Lepidosaphes piperis* while fish oil rosin 3% was more effective against *A. destructor* in black pepper.

Social Sciences

The Section of Social Sciences links the research institute with growers, developmental agencies and industry. It carries out analysis of agrobiological data, studies the socioeconomic problems in transferring technologies to farmers and conducts demonstrations, trainings, exhibitions, etc.

Demonstration trials

Black pepper production was increased from 0.5 to 1.3 Kg vine^{-1} and the incidence of foot rot disease was brought down to 0.8 % from 2.5 % in a coconut + pepper plot by the adoption of HPT. Adoption of HPT in seven farmers' plots with

different cardamom based cropping systems showed that the yield of cardamom can be substantially increased.

IISR adopts Kodenchery Panchayat

Kodenchery Panchayat was adopted by the IISR, Calicut for its all-round development. A seminar and exhibition were organised from 14-15 October 1996. Sri. V.K. Rajan, Minister for Agriculture, Government of Kerala inaugurated the seminar and exhibition, in which various developmental agencies and non governmental organisations participated. Lectures were delivered on various topics of public interest viz., agriculture, animal husbandry, horticulture, floriculture and vegetable cultivation. As the follow up action, a benchmark survey was conducted in the adopted villages and the major problems of the farmers were identified.

Training programmes

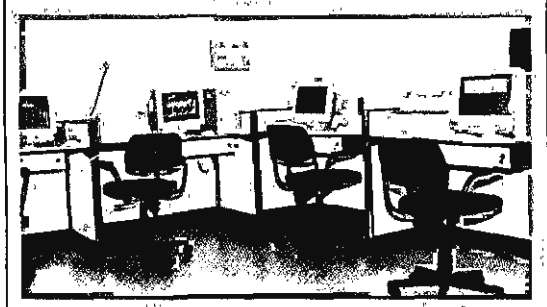
Four training programmes on "Spices production technology" (thrice), "*Phytophthora* foot rot disease management in black pepper" (twice), "Nursery management in spices" and "On farm processing of spices" (once each) were conducted where in 74 officers from different states had participated. "A field training on spices" was organised for 17 B.Sc. (Ag.) final year students of Allahabad Agriculture Institute, Allahabad, Uttar Pradesh.

Mr. U.K.S. Chauhan IAS, District Collector, inaugurating the seminar cum exhibition organised on the occasion of Kodenchery village adoption by IISR

Exhibitions

The institute conducted an exhibition at Kodenchery from 14-15, 1996 on the occasion of Kodenchery village adoption programme. Besides, it also participated in five exhibitions on different occasions viz., 1. EURECCA 96 (Science & Technology exhibition), conducted by Regional Engineering College at Zamorin school, Calicut: 5-12 April 1996. 2. 40th anniversary of Kaiveli panchayat organised by NWDPRA, at Kaiveli, Calicut: 13-14 September 1996. 3. 40th anniversary of St. Joseph's College, Devagiri, Calicut: 4-5 December 1996. 4. 40th anniversary of Chathamangalam Panchayat, Calicut: 22-25 December 1996. 5. 40th anniversary of Cheruvady Panchayat, Calicut: 27-30 December 1996.

ARIS Cell

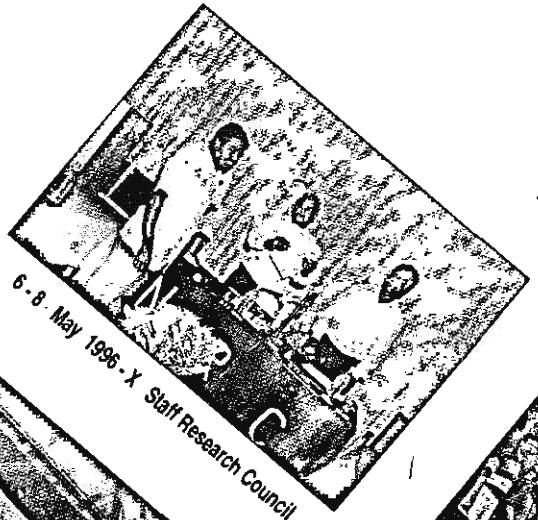


An Agricultural Research Information System (ARIS) cell has started functioning at IISR, Calicut under the Social Sciences section.

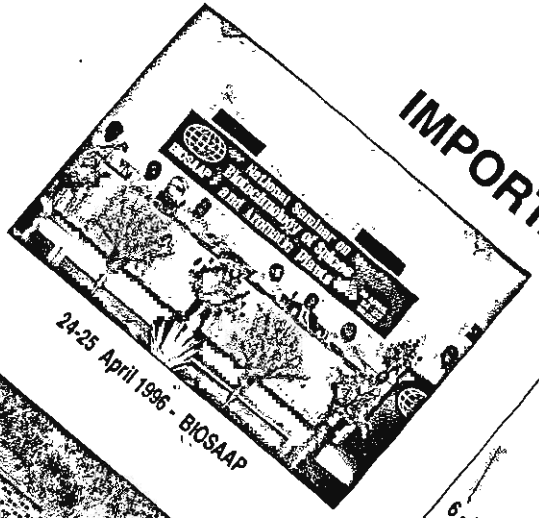
Krishi Vigyan Kendra

During the period, KVK conducted 58 short duration training programmes. Altogether 1975 trainees participated in these training programmes. In the Plant and Animal Health Centre, a total of 1730 cases was treated. An artificial insemination centre was also started under the centre during the period. Under the revolving fund scheme an amount of Rs.91,357 was generated by way of sale of planting materials and as consultation and registration fees.

IMPORTANT EVENTS



6 - 8 May 1996 - X Staff Research Council

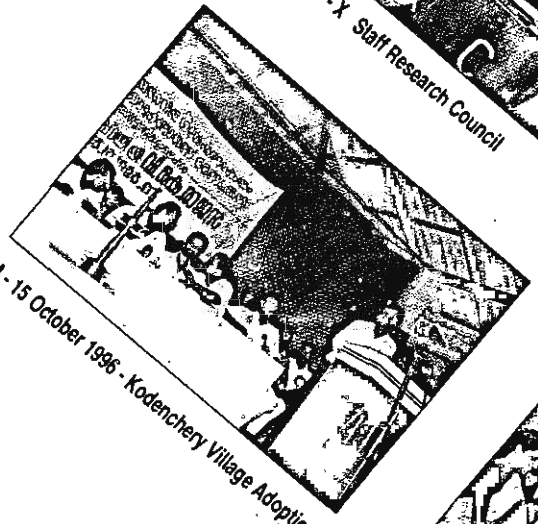


24-25 April 1996 - BIOSAAP

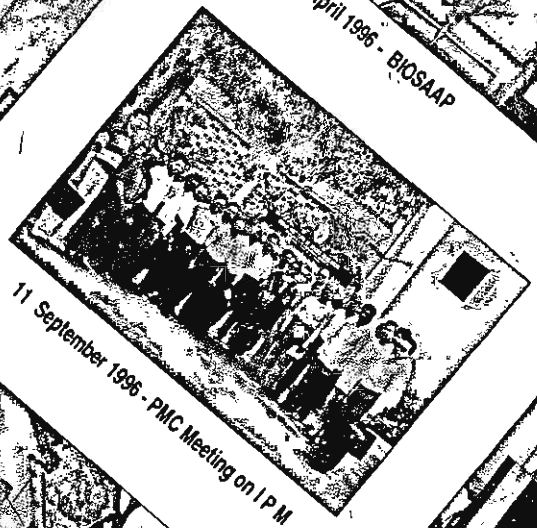
29 May 1996 - Visit of Dr.M.S.Swaminathan

1 July 1996 - I I S R Annual Day

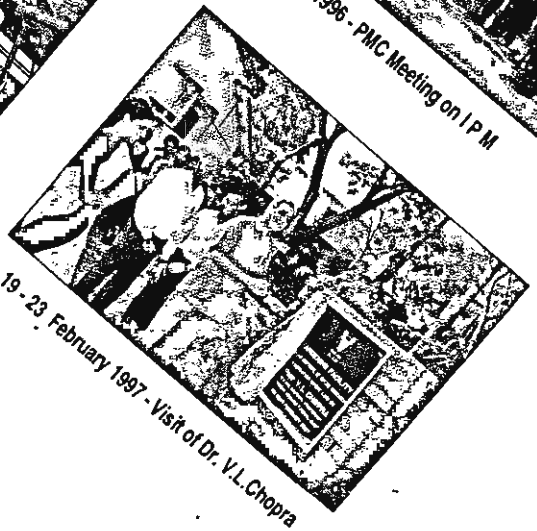
6 - 7 November 1996 - Mid Term Review Meeting



14 - 15 October 1996 - Kodenchery Village Adoption

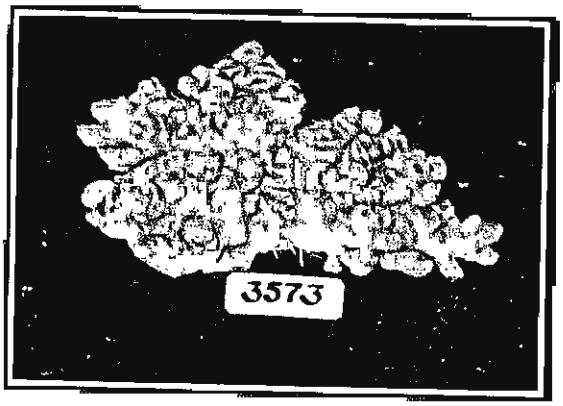


11 September 1996 - PMC Meeting on I P M



19 - 23 February 1997 - Visit of Dr. V.L.Chopra

11 - 12 October 1996 - Interface with Spices Exporters



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