

Research Highlights

2005–06



Indian Institute of Spices Research
(Indian Council of Agricultural Research)
Calicut, Kerala, India

Correct citation

Indian Institute of Spices Research, Research Highlights 2005–06, Calicut

Publisher

Director

Indian Institute of Spices Research

Calicut – 673 012, Kerala, India

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May 2006

Printed at

Printers Castle, Kochi

Cover page: Indian Cardamom : Superior in quality (p. 9)



PREFACE

The Research Highlights for 2005-06 of the Indian Institute of Spices Research is a comprehensive presentation of the summary of achievements made by this institute during the year. The institute has made commendable contribution in various frontier areas of research besides concentrating on development of technologies for the farmers. Our strength has always been the large amount of genetic diversity being conserved in the institute. The diversity of genetic resources encompasses a large collection of microbes in addition to various spice crops. The tough and eluding diseases such as foot rot in black pepper and the emerging viral diseases continued to draw our attention and we are on the threshold of developing diagnostics. Use of weather parameters for forecasting yield in black pepper is underway. We also found that the cardamom from India is better than the cardamom from Gautemala and Sri Lanka. Research on ginger and turmeric has indicated the presence of bacterial wilt resistance in a Zingiberaceae species, *C. amada*. Our research is also directed towards the control of mycotoxins. The All India Coordinated Research Project on Spices being carried out at 19 centres has identified a few varieties for release in the AICRP Group Meeting to be held in May 2006. I place on record our gratitude to Dr. Mangala Rai, Director General and Dr. Gautam Kalloo, Deputy Director General for their wise counsel. I also thank Dr. K V Ramana, Assistant Director General (PC), Dr. S Kannaiyan, Chairman (Research Advisory Committee) and members of RAC for their helpful suggestions.

I thank the Editorial Committee consisting of Dr. A Kumar, Scientist and Secretary, Staff Research Council, Dr. A Ishwara Bhat, Senior Scientist, Dr. R Dinesh, Senior Scientist, T.E. Sheeja, Scientist and Dr. Utpala Parthasarathy, Technical Officer for bringing out the Research Highlights in an excellent manner.

Calicut

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CONTENTS

	Page
I Black Pepper	5
II Cardamom	9
III Ginger and Turmeric	10
IV Tree Spices	13
V Vanilla	13
VI Paprika	14
VII AICRP on Spices	14



I. Black pepper

GENETIC RESOURCES

A total of 62 accessions were added to the germplasm repository through surveys conducted in Chimmony and Parambikulam Wild Life Sanctuary (Kerala), Nelliampathy forests (Kerala), Cherapunji (Assam), Wayanad (Kerala) and Tirupathi (Andhra Pradesh).

A male *P. galeatum* with spike length of 24.5 cm from Nelliampathy forest was found to be a unique collection for its unusually long spike.

Bio-climatic analysis and prediction system (BIOLIM) comparison of 16 wild species of *Piper* occurring in South India indicated the rich diversity and prospective 'niches' where *Piper* species occur predominantly. The highest richness grid was found to have 15-16 species while the highest diversity value was found to range from 1.8 to 3.

GENETIC IMPROVEMENT

Earliness in flowering was observed in micropropagated black pepper plants as compared to normal plants when it was evaluated in 87 field trials in Kerala and Karnataka covering 23 ha.

Accession number 5479, a local collection from Mananthavady, Kerala was found to be very high in caryophyllene (21.75%) and bulk density (640 g L⁻¹)

DNA profiling studies on different *Piper* species indicated that *P. nigrum* is very close to *P. sugandhi*, *P. bababudani*, *P. schmdtti* and *P. wightii* were also found to be related to *P. nigrum*. Longum types formed a separate

subgroup in the phylogenetic classification. Variation in the inter-microsatellite regions among cultivars was found to be low.



P. sugandhi a close relative of *P. nigrum*

Gene for resistance against *Phytophthora capsici*

Isolation and sequencing of the part (internal region) of the resistance gene was achieved by targeted gene amplification using degenerate primers. The sequence consisted of 252 bp (base pairs) encoding for 84 amino acids. Sequence comparison of the deduced amino acid sequences showed sequence similarity with already identified disease resistance genes in public databases.



PEST AND DISEASE MANAGEMENT

Phytophthora foot rot

Resistance

Fourteen wild accessions of *Piper* spp, thousands of open pollinated seedlings from 27 accessions, another 35 promising open pollinated seedlings and hybrids were screened for their reaction to *Phytophthora capsici* using leaf, stem and root inoculation methods. Among them, one seedling each designated as P-24 (04-P24-1) and HP-1533 (04- HP 1533-1) showed tolerant reaction to *Phytophthora* infection in leaf, stem and root.

Management

Evaluation of black pepper on *P. colubrinum* rootstock (resistant to *P. capsici*) indicated that the grafts remained healthy even after seven years of planting and an average yield of 0.650 kg (dry) pepper per vine was obtained in un-irrigated gardens.

Viral diseases

Molecular characterization of badnavirus

Sequence analysis and comparison of portion of Open Reading Frame I (ORF I) and ORF III of badnavirus infecting black pepper with other known badnaviruses, indicated high levels of identity with *Piper yellow mottle virus* (PYMV) followed by *Banana streak virus* (BSV).

Development of diagnostics

A method for simultaneous isolation of RNA and DNA from infected black pepper plants and multiplex PCR for simultaneous detection of

***Cucumber mosaic virus* and Badnavirus in a single reaction was standardized.**

Nematodes

Resistance

Among the nine short-listed nematode resistant black pepper lines, Acc. 820 (IC No. 316481), Acc. 1090 (IC No. 316635) and HP-39 were healthy and free from nematode infestation after two years of planting. HP-39 and Acc. 1090 were rich in caryophyllene content. Acc. 820 was registered with NBPGR, New Delhi.

Diagnostics for *Radopholus similis*

Species-specific primers were developed for the identification of *R. similis* based on sequence information from the ITS gene, and their use in identifying *R. similis* was proved. The primer set generated a single PCR fragment of ~400 bp in length that was specific to *R. similis*.

Management

Nematode populations and the consequent yellowing were significantly less in plots where bacterial antagonist, IISR-859 was applied along with biocontrol consortia.

Promising bacterial antagonists against nematode pest of black pepper

IISR-8, IISR-151 and IISR-859 were compatible with *Trichoderma harzianum*. Interestingly IISR-859 was compatible with nematode antagonist, *Pochonia chlamydosporia* too.

Based on morphological and biochemical characterization, 109 isolates of putative en-



cess rainfall during initial period (i.e. between 5-11th March to 25th June-1st July) and terminal period (Beyond 1st December) of annual cycle is detrimental to yield whereas excess rainfall between July to December end was beneficial to crop and would help in enhancing the yield.

Granite powder - a substitute for sand in black pepper nursery mixture

Black pepper is propagated through rooting of excised shoots using growth medium consisting of soil, sand, and farmyard manure in 2:1:1 proportion. Substituting the sand with granite powder, a waste material obtained from stone quarries, in the above medium was found to produce increased rate of leaf produc-

tion (4.6), leaf area (136.8 cm²), and biomass (3.9g) in black pepper rooted cuttings.

PHYSIOLOGICAL CHANGES UPON DROUGHT

In black pepper accessions photosynthetic rate, transpiration rate and stomatal conductance decreased drastically within six days of stress, whereas the leaf temperature increased. Photosynthetic rate decreased from 1.91 to 0.6 μ moles while transpiration rate decreased from 1.11 to 0.24 μ moles and stomatal conductance decreased from 0.07 to 0.004.

POST HARVEST TECHNOLOGY

The berries belonging to five varieties of black pepper were graded to 4 sizes i.e. <3.5mm, 3.5 to 3.8mm, 3.8 to 4.8mm and



Granite powder- a substitute for sand in black pepper nursery mixture



>4.8mm. More than fifty percent of the berries were of the size 3.5 to 3.8 mm.

Vacuum package for Black Pepper

The black pepper samples stored in vacuum for about eight months showed good quality indicating the suitability of storage in vacuum containers for long shelflife.

High quality pepper in organic system of cultivation

Essential oil constituents of organically cultivated black pepper showed higher values of caryophyllene in Panniyur-1 (30%) and Karimunda (24%).

Out of the five black pepper samples collected from Munnar, HP-105, HP-728 and C-1041 contained 4% oil, while oleoresin ranged from 7.8-10.5% and piperine 1.6-2.9%. Essential oil from HP-34 contained 18.5% caryophyllene followed by HP-105 with 15% caryophyllene.

Microbial fermentation for production of white pepper

Among the 52 microorganisms screened for microbial decortication of black pepper, eight of them were found to be promising with conversion percentage of over 60%. Optimum fermentation temperature for microbial conversion into white pepper was found to be 35°C.

II. Cardamom

GENETIC RESOURCES

A total of 23 cardamom germplasm collections were made from cultivated and forest

areas of Kerala. Flowers with completely white labellum from Idukki and bold capsule accessions from Parambikulam were found to be unique. A total of 416 accessions have been maintained in the field germplasm repository and 72 of them were characterized (13 compound panicle and 59 Malabar accessions) for 15 quantitative characters based on IPGRI descriptor.

GENETIC IMPROVEMENT

APG 293 (1045 kg/ha), APG 398 (1498 kg/ha), APG 416 (1487 kg/ha) and compound panicle type APG 250 (1770 kg/ha) were found to be high yielding genotypes

Indian Cardamom- superior in quality

Physical and biochemical characterization of traded cardamom from India, Sri Lanka and Guatemala revealed the superiority of Indian produce for the physical parameters such as seed to husk ratio, weight of 100 capsules, capsule count in 100 g produce, bulk density and moisture content. GC profile of cardamom oil revealed high 1, 8-cineole and α terpinyl acetate in the Indian cardamom.

PEST AND DISEASE MANAGEMENT

Eight high yielding accessions among the 23 elite accessions showed moderately resistant reaction to the foliar diseases, leaf blight (*Colletotrichum gloeosporioides*) and leaf blotch (*Phaeodactylum alpiniae*)

Management

Evaluation of consortia of biocontrol agents



for the management of rhizome rot disease indicated that root rot and mortality were less with *Trichoderma* alone (MTCC 5179) followed by consortium of biocontrol agents (IISR-6, IISR-13, IISR-51, IISR-151, IISR-853 and IISR-859) and copper oxychloride @ 0.2 %.

DROUGHT TOLERANCE IN CARDAMOM

Cardamom genotypes APG 257, APG 414 and APG 434 with higher biomass and higher relative water content under stress were adjudged to be relatively tolerant to moisture stress.

QUALITY EVALUATION IN CARDAMOM

Among the seventy-one germplasm samples analyzed, NHY-15, RR-1xMB-3, MA-7, VA-1, and WC contained 6% oil and NHY-14, MB-3, NHY-18 and OP-28 possessed above 40% α - terpinyl acetate in the oil.

III. Ginger and Turmeric

GENETIC RESOURCES

A total of 625 accessions of *Zingiber* and 924 accessions of *Curcuma* are maintained in the *ex situ* gene bank.

Suitability Studies

GIS studies indicated that location suitability is an important factor to determine the productivity of the crop. The GIS based suitability map further revealed that Andhra Pradesh is highly suitable for turmeric cultivation leaving a small patch in the central and western part. The area production curve for 30 years when compared with the suitability map showed correlation to a great extent.

GENETIC IMPROVEMENT

Evaluation of selected Nepal accessions of ginger revealed the superiority of Acc.578 (13.1 kg fresh rhizome 3m² bed⁻¹) with low fiber (2.8%). Among the 13 high oil type ginger lines evaluated for yield, Acc.162 recorded maximum yield of 10.1 kg 3m² bed⁻¹ with 2% oil.

Efficient protocol for plant regeneration from callus through organogenesis and somatic embryogenesis was standardized in turmeric on MS supplemented with 0.5 μ M TDZ and 0.1 μ M TDZ respectively. Variations in rhizome morphology were observed among callus-regenerated plantlets.

PEST AND DISEASE MANAGEMENT

Bacterial wilt and Rhizome rot diseases

Etiology

Isolates of *Ralstonia solanacearum* causing bacterial wilt of ginger in North Eastern States, Sikkim and Kerala were found to have 100% similarity coefficient in rep-PCR profile indicating strain migration from one location to another.

Resistance

Ginger strain of *R. solanacearum* was found to infect turmeric, cardamom, *Curcuma aromatica*, *C. zedoaria*, *Kampferia* sp., *Zingiber zerumbet* and tomato but not *C. amada*. Mango ginger (*C. amada*) was found unaffected by the ginger bacterial wilt pathogen *R. solanacearum*. Interestingly the plant wilted when the pathogen was inoculated into pseudostem after making a pinprick indicating that the bacterium could not make entry into the plant system through roots. Besides





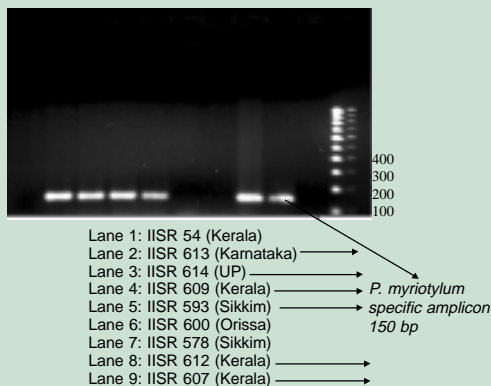
Curcuma amada- A bacterial wilt evading species in Zingiberaceae

Inset: Rhizomes of *C. amada*,
 Foreground: Wilting of *Z. officinale*,
 Background: Surviving *C. amada*

R. solanacearum was found to survive on soil, roots, and rhizome of mango ginger without causing any yield reduction.

Identification of *Pythium*

The species of *Pythium* causing rhizome rot of ginger in Kerala, Karnataka, Uttar Pradesh and Sikkim was identified as *P. myriotylum* by PCR based method. Primers specific for *P. myriotylum* were found to amplify 150 bp sequences in the genomic DNA of *P. myriotylum*.



Management

Integrated management trial on turmeric rhizome bacteriazation revealed that a consortium of bacteria yielded significantly higher rhizome with 14.6 t ha⁻¹ when compared to other treatments.

Nematodes

Resistance

Resistance of six turmeric accessions shortlisted earlier viz., Accs. 57, 62, 72, 150, 193 and 199 was confirmed by repeated inoculations.

Pests

Shoot borer (*Conogethes punctiferalis* Guen.) damage and its distribution in turmeric

Apart from boring into the main pseudostem at the base, the shoot borer larva was found to enter into the main central shoot by damaging the young leaf petiole. The symptoms of pest infestation were first observed during August and maximum new infestations occurred during October to November. The pattern of pest distribution was random up to September and became more aggregated during October, November and December.

Host resistance

Screening of 895 accessions of turmeric maintained in the Germplasm Conservatory for the incidence of shoot borer (*Conogethes punctiferalis*) showed that two accessions were free of infestation.

Natural enemies

Mermithid nematodes and dermapterans were recorded as natural enemies of shoot borer larvae in the field. Mermithid nema-



Early symptom of shoot borer larvae infestation on tender leaf of turmeric



Symptom of entry of shoot borer larvae into central shoot of turmeric through leaf petiole

todes were observed throughout the crop season and the percentage of population of larvae parasitised by the nematode was higher during August and September.

NUTRIENT MANAGEMENT

A highly significant positive correlation of leaf P/Zn ratio and a significant negative correlation of soil P/Zn ratio with rhizome yield of ginger were observed. Step wise regression analysis showed that leaf P/Zn is most influencing factor on rhizome yield followed by soil P/Zn ($r^2 = 0.353^{**}$). The critical range of leaf P/Zn ratio was found to be 27.9 to 90 through Mitscherlich model.

QUALITY EVALUATION

Accs-277, 399, 408, 537 (Kozhikkalan) and Acc-246 (Sabarimala) showed more than 5.5% oleoresin. The exotic accession of ginger, *Zingiber officinale* var. *rubens* ('Kintoki') analysed for quality features indicated 1.27% fiber, 1.5% oil, 3.35% oleoresin, 52.57 % starch and 43.8 % dry recovery. Turmeric Acc-1, 25, 35, 21 and 50 were found to contain more than 20% oleoresin. Accessions with high oil were 62, 1, 47, 35, 39, 48, 20, 15, 32, 10, 2, 50 and 23 (more than 8%). Acc-50 contained 7.2% curcumin with 21% oleoresin.



GC MS analysis of ginger and turmeric oil

GC MS analysis of ginger oil indicated the presence of citral-a: 5.3%, citral-b: 8.1%, ar-curcumene: 7.8%, α - zingiberene:19.1%, β - bisabolene: 7.5%, α - farnesene: 6.6%, β - sesquiphellandrene:10.2%. GC MS analysis of turmeric rhizome oil indicated the presence of ar-curcumene: 1.5%, α -zingiberene: 1.45%, β - sesquiphellandrene: 1.6%, ar-turmerone: 28.47%, β -tumerone 9.2%, ar-turmerone: 3.1% and α - tumerone: 22.17%.

IV. Tree spices

GENETIC RESOURCES

IC Numbers for clove, nutmeg and all spice accessions were obtained from NBPGR, New Delhi. Cassia C1 (IC 370415) has been registered as INGR 05029 with NBPGR, New Delhi for its high oleoresin content (10.5%). Ten accessions of *Garcinia gummigutta* and *G. cowa* from Kerala and one *Syzygium* sp. from Cherapunji (Meghalaya) were collected during the year.

GENETIC IMPROVEMENT

In *Garcinia*, the clone of a very high yielding and late maturing type collected in 2000-01 had flowered in 3rd year after planting in farmer's field with the fruits maturing in September-October.

Nutmeg

Protocols for establishing sterile cultures were standardized. Nodal explants showed bud break from leaf axils in 3% cultures in SH media. Better survival of cultures was obtained on SH supplemented with 2mg/l BAP.

Evaluation of *Cinnamomum* species for leaf oil constituents

Six *Cinnamomum* species namely *C. verum*, *C. cassia*, *C. camphora*, *C. tamala*, *C. citriodorum* and *C. malabratrum* were evaluated for leaf oil constituents. The major constituents were identified as eugenol (87%), cinnamyl acetate (39%), camphor (59%), eugenol (32%), citronellol (42%) and linalool (32%).

POST HARVEST TECHNOLOGY

Nutmeg

Using hot air drier, drying of mace took 3½ h at 60° C, while nut took 15 days. The dry recovery of mace was 35.6% and that of nut was 58.2%. The ratio of nut to mace recovery was 5.75:1. Mace blanched for two minutes in boiling water showed better colour retention (Lycopene-49.9%) than 'one minute blanching' (Lycopene 38.7%) and 'no blanching' (Lycopene- 9.98%) even after 180 days of storage in 300-micron polypropylene containers.

V. Vanilla

GENETIC RESOURCES

Accessions of wild *Vanilla* species from Andaman and Nicobar Islands produced flowers of different colours in different branches of the same plant. Purplish pink and light pink flowers were produced by Acc 4704A and 4706 while purplish pink and pure white flowers were produced by Acc 4709 in different branches. Accessions 4705, 4707 and 4708 continued to produce purplish pink flowers and 4704B produced pure white flowers.



PEST AND DISEASE MANAGEMENT

Survey for diseases

Root rot and wilt were found to be the major problem in most of the plantations. Root rot incidence ranged from 5 to 100%. Mosaic and necrosis were also observed in all the plantations and the incidence ranged from 2 to 80%.

Etiology

A virus causing mild chlorotic mottle and streaks on leaves of vanilla was identified as a strain of *Cymbidium mosaic virus* (CymMV) based on coat protein (CP) gene sequence comparison and phylogenetic studies. An identity of 92.3% to 97.3% was seen with different CymMV isolates infecting different orchids in India while with available partial CP sequences of CymMV isolates infecting vanilla, identity ranged from 98.2 to 99.4%.

VI. Paprika

GENETIC RESOURCES

A total of 57 indigenous and 11 exotic collections/accessions of paprika and paprika like chillies have been collected from various sources and added to the existing germplasm, raising the total to 130.

The color value of 85 selected lines was analyzed, which ranged from 110 to 354 ASTA color units, the highest being with ICB-19 (354 ASTA units) followed by ICB-11 (348 ASTA units). Acc 0107-7011 showed 308 ASTA units with 15.9% oleoresin and 1.02% capsaicin. MS-2X B-2 showed 308 ASTA units with 15.7% oleoresin and 1% capsaicin.

TOXIGENIC FUNGI ON SPICE PRODUCE

Analysis of 93 spice samples obtained from markets in Andhra Pradesh and Kerala, revealed that *A. niger* was predominant, followed by *A. flavus*, *A. parasiticus* and *Penicillium* sp.

VII. All India Coordinated Research Project on Spices

GENETIC RESOURCES AND IMPROVEMENT

Genetic resources at the AICRPS centers consist of 612 accessions of black pepper, 273 of cardamom, 603 in ginger, 1332 in turmeric, 202 in tree spices and 3961 in seed spices. In total eighty-six lines for yield and 36 lines for quality attributes were identified through CVT/CYT/IET/germplasm evaluation.

Produce	Percentage of samples infected with fungi			
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus parasiticus</i>	<i>Penicillium</i> sp
Black pepper	56.0	8.0	16.0	4.0
Ginger	75.0	8.3	2.5	-
Turmeric	100.0	8.3	-	-
Nutmeg	78.0	5.2	10.0	5.2



BIOFERTILIZERS AND TRADITIONAL NUTRITION FOR SPICES

Supplementing biofertilizers, *Azospirillum* (50g) and phosphobacteria (50g) separately in combination with recommended inorganic fertilizer enhanced the yield of black pepper, cardamom, ginger and turmeric. Addition of traditional nutrient sources like burnt earth (10.0kg) and wood ash (2.0kg) along with Farm Yard Manure (10.0kg) was promising in increasing black pepper yield (6.4kg/vine).

NUTRIENT SCHEDULE FOR GINGER AND TURMERIC

Recommended dose of fertilizers registered maximum yield of 17.9 t/ha in ginger and 26.0 t/ha in turmeric at Raigarh; 18.7 t/ha in ginger and 18.3 t/ha in turmeric at Pottangi; 25.0 t/ha in turmeric at Pundibari. Application of FYM 10kg+ Pongamia oil cake+Neem oil cake+Sterameal + Rock phosphate + wood ash, 250g each/bed recorded higher yield of 23.7 t/ha in ginger at Dholi. Application of zinc sulphate @ 25.0 kg/ha produced maximum ginger yield (20.3 t/ha) at Dholi.

LOCATION SPECIFIC NUTRIENT SCHEDULE FOR SEED SPICES

In coriander, micronutrient spray viz., $ZnSO_4$ + $FeSO_4$ + $CuSO_4$ + $MnSO_4$ (each at 0.5%) recorded the higher yield (940 kg/ha). Application of 100% inorganic N + *Azospirillum* @ 1.5 kg/ha + 5 t FYM/ha resulted in maximum seed yield in coriander (3.5 t/ha), cumin (323 kg/ha) and fennel (1.2 t/ha), whereas 100% inorganic N alone gave the highest yield (1.3 t/ha) in fenugreek at Jobner

Centre. However, maximum yield was obtained in coriander, fennel and fenugreek with the application of 10t/ha of FYM + 1.5 kg/ha of *Azospirillum* as seed treatment at Kumarganj (U.P.) conditions. At Coimbatore (T.N.) conditions, application of FYM 5t/ha + *Azospirillum* 1.5 kg/ha as seed treatment along with inorganic N 50% and 100%, produced the highest yield in coriander (738 kg/ha) and fenugreek (685 kg/ha), respectively. In coriander, spray of bioregulator, Triaccontanol @ 0.5%, thrice at 40, 60 and 80 days after sowing significantly increased the yield (1.5 t/ha) at Dholi, while highest yield (1.5 t/ha) was achieved with spraying of Triaccontanol @ 1.0%, thrice at 40, 60 and 80 days after sowing at Kumaraganj. However, maximum yield of 1.0 t/ha was obtained with spraying of NAA 10 ppm, twice at 40 and 60 days after sowing at Guntur.

PEST AND DISEASE MANAGEMENT IN SPICE CROPS

Foot rot in black pepper

Planting of black pepper cuttings in solarized soil fortified with *Trichoderma harzianum* @ 1g/kg soil and VAM inoculum @ 100 cc/kg soil was found ideal for production of healthy rooted cuttings at Chintapalle and Pampadumpara centers. Application of Metalaxyl Gold MZ (2.5 g l⁻¹) and *T. harzianum* was effective for controlling foot rot disease in the field at Panniyur centre. However, at Mudigere centre, spraying and drenching with Bordeaux mixture (1%) during May/June and July-August was most effective. At high altitude areas, maximum reduction of anthracnose disease in black pepper could be achieved by spraying carbendazim + mancozeb 0.1% as foliar



