

# Research Highlights 2016-17

ICAR-Indian Institute of Spices Research (Two times winner of Sardar Patel Outstanding ICAR Institution Award) Kozhikode



#### Publisher

K. Nirmal Babu Director ICAR-Indian Institute of Spices Research Kozhikode

#### **Editorial Board**

D Prasath R Dinesh KS Krishnamoorthy CM Senthil Kumar Lijo Thomas

May 2017

#### **Correct Citation**

Prasath D, R Dinesh, KS Krishnamoorthy CM Senthil Kumar, Lijo Thomas (Eds) (2017) Research Highlights 2016-17, ICAR Indian Institute of Spices Research, Kozhikode Kerala, India, p. 24.



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The highlights of research achievements of the institute during 2016-17 are presented in this publication. During the year, 33 *Piper* accessions including 31 cultivars and two wild types were collected. The alternate field gene banks for black pepper are maintained at CHES, Chettalli, Karnataka and IISR, Chelavoor campus, Kerala. The significant achievement during the year was development and release of a high yielding, short duration and stable curcumin turmeric variety, IISR Pragati. This variety paves the way for curcumin upgradation in major turmerie growing states. Three new accessions of nutmeg with bold nut and thick mace were added to the germplasm

Large scale demonstrations on site specific nutrient management in black pepper showed significant increase in yield as compared to farmers practice. Studies on *in vitro* cytotoxic effect of spice extracts on the cell lines SKBR-3 and HCT-116 indicated highest cytotoxic effect in hexane extract of cinnamon.

IPM strategy was developed for the control of cardamom thrips *i.e.* two sprays of spinosad 0.0135% and soil application of the entomopathogenic fungus, *Lecanicillium psalliotae*. In ginger, soil solarization followed by application of 3% calcium chloride was found to be effective in bacterial wilt management.

The advisory services of the Agricultural Technology Information Center were delivered to more than 2700 clients. Thirteen training programmes were conducted by the institute targeting diverse stakeholder groups like farmers, youth, tribal beneficiaries and students. A total of 48 exhibitions were organized during the year. In KVK, about 112 training programmes for practicing farmers and farm women, rural youth and extension functionaries were conducted. Eight front line demonstrations and seven on farm trials on technology assessment and refinement were carried out. During the year, the ITM-BPD Unit facilitated seven non exclusive licensing of black pepper, turmeric and ginger varieties. License agreement for cleaning, grading, packing and powdering of black pepper and white pepper was signed with CAMPCO Pvt India Ltd.

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C Pr Cl (E In Ke Overall, the efforts of the scientists, staffs, associates, farmers, planters and entrepreneurs have been unprecedented, overwhelming and encouraging. Your releatless support and fair appraisal are what give me the verve to strive for betterment and strengthening of our research and extension programmes to trigger the change we want to witness in the spices sector.

I consider it a privilege to place on record the encouragement given by Dr. T. Mohapatra, Secretary, DARE and Director General, ICAR and Dr. S. Ayyappan, former Secretary, DARE and Director General, ICAR. I am also grateful for the strong support and necessary guidance received from Dr. A. K. Singh, Deputy Director General (Hort. Science), Dr. N.K. Krishna Kumar, former Deputy Director General (Hort. Science) and Dr. T. Janakiram, ADG (Hort. Science). I appreciate the efforts and zeal shown by all the project investigators in executing various programmes. The financial support received from ICAR is gratefully acknowledged. I also appreciate the editors for having compiled and brought out this publication.

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(K. Nirmal Babu)

Kozhikode, 19.05.2017

## **BLACK PEPPER**

#### **Genetic Resources**

Thirty one cultivated accessions and two wild accessions from Arunachal Pradesh (Fig.1) were collected. The unique accessions collected from Kodagu district, Karnataka are:

- A accession with very long spike (about 30 cm long)
- An accession having male, female and bisexual flowers in the same vine
- A accession with very less pungent ('sweet') berries
- A variant resembling the triploid "Vadakkan" but with elongated leaves.

A total of 3395 black pepper accessions are now maintained at *ex situ* conservatory. A field gene bank of 200 accessions at Peruvannamuzhi, 223 at Chelavoor and 627 accessions at the alternate field gene bank at CHES, Chettalli are also being maintained. The alternate field genebank at CHES, Chettalli was enriched with 100 accessions. Eighty five accessions were characterized based on IPGRI descriptors for 27 traits.

#### Breeding

In a trial involving 10 improved genotypes, maximum fresh yield vine<sup>-1</sup> was recorded in Hp 117 x Thommankodi (3.46 kg vine<sup>-1</sup>, 30.2% dry recovery) (Fig. 2) followed by OPKM (2.679 kg vine<sup>-1</sup> with 33.2% dry recovery) and Thevam (2.49 kg vine<sup>-1</sup> with 31.3% dry recovery). Quality wise, Sreekara (control) and Hp 728 had 3.6% oil each followed by Hp 1411 and Coll. 820 with 3.5 and 3.4% oil, respectively. However Coll. 820 recorded highest oleoresin (10.2%) followed by Hp 1411 (9.1%) and Hp 728 (9.0%).



Fig. 1. Mature spike of *Piper* species collected from Arunachal Pradesh

#### Transcriptome analysis

Transcriptome sequencing was performed using drought tolerant Acc. 4226 exposed to water deficit stress. Sequencing using the PacBio protocol resulted in 53689 reads of insert (ROIs) with mean read length of 2378 bp. The transcripts were aligned against the drought responsive gene sequences in the drought stress gene database (Drought DB) and 431 transcripts were found matching to molecular adaptation regulatory (MAR) sequences, 96 were aligned to molecular adaptation functional (MAF) sequences and 331 were aligned to physical adaptation (PA) sequences.

Fig. 2. High yielding hybrid, Hp 117 x Thommankodi



## Mining of antimicrobial peptides (AMP) from transcriptome

A cysteine rich peptide showing similarity to plant defensins with a mature peptide length of 48 amino acids was discovered from the derived transcripts.

#### **Crop Management**

#### Scheduling fertilizer dose for fertigation

An adaptive trial was conducted at Laxmi Estate, Mudigere, Karnataka. Significantly higher yield was registered with foliar supplementation of NPK (5.42 kg std<sup>-1</sup> fresh) and NPK + micronutrients (5.36 kg std<sup>-1</sup> fresh). Additional investment of Rs. 15.00 per standard has yielded an additional profit of Rs. 175.00 per standard.

### Management of virus affected gardens for yield sustainability

Trials on the management of virus affected black pepper gardens were taken up in three locations at Madapura, Chettalli and Pollibetta in Karnataka. The fresh yield was higher in application of FYM + NPK + PGPR + micronutrients (3.6-7.1 kg std<sup>-1</sup>) which was on par with FYM + NPK + micronutrients application (3.2-6.4 kg std<sup>-1</sup>) and significantly higher than control (2.4-4.4 kg std<sup>-1</sup>) across locations.

#### Promising Zn solubilising bacteria

Six promising strains (ZSB1-*Burkholderia* sp.; ZSB2-*Bacillus megaterium*; ZSB3- *Lysinibacillus* sp.; ZSB4-*Bacillus* sp.; ZSB5-*Burkholderia* sp and ZSB8-unidentified) were further tested for their Zn solubilization efficiency in liquid medium with reference to the Zn solubilising isolate *Gluconaceto-bacter diazotrophicus* (PAL-5), obtained from TNAU, Coimbatore, Tamil Nadu (Fig. 3). Marked differences were observed in the Zn solubilization efficiency at different days after initiation (DAI) and the most promising bacteria was ZSB2 (*Bacillus megaterium*; NCBI-KY687496), registering as high as 126.8 mg L<sup>-1</sup> Zn at 7th DAI.



Fig. 3. Zn solubilization efficiency (mg L<sup>-1</sup>) of promising bacteria isolated from soils under wild black pepper and cardamom at different days after initiation (DAI) [C-Control; ZSB1- *Burkholderia* sp.; ZSB2-*Bacillus megaterium;* ZSB3- *Lysinibacillus* sp.; ZSB4-*Bacillus* sp.; ZSB5-*Burkholderia* sp; ZSB8-unidentified; PAL5- *Gluconacetobacter diazotrophicus*]

#### Delineation of spices zone

Efficient cropping zones of black pepper were identified based on the criteria of relative yield index (RYI) and relative spread index (RSI). In Kerala, Kasaragod is the efficient black pepper producing zone with high RYI of 147, Idukki and Kozhikode are efficient zones with little year to year variation. In Karnataka, Chikkamagaluru is the most efficient zone, whereas, Dakshina Kannada, Hassan, Kodagu, Shivamogga, Udupi and Uttara Kannada are efficient zones with little year to year variation. In Tamil Nadu, Dindugul is the most efficient zone and The Nilgiris, Namakkal and Salem are efficient zones with little year to year variation.

#### Drought management in black pepper

Among the antitranspirants tested (Kaolin 1.5% and 2%, spray lime 1.5% with 0.5% SOP), black pepper vines treated with Kaolin 2% spray showed higher photosynthetic rate combined with minimum transpiration rate (i.e. minimum water loss) and lower leaf surface temperature.





Steam blanching of green pepper in the solar curing unit Blanching of black pepper for 4 min with steam generated from the solar unit (Fig. 4) ensured complete drying in 4 days and registered higher essential oil content compared to dipping in boiling water for 1 min and control (unblanched pepper).

#### **Plant Health Management**

#### Diversity of Phytophthora

A host range study comprising of four different host *viz.*, black pepper, pothos, chilli and capsicum was carried out using 18 selected black pepper isolates of *Phytophthora* and compared with ATCC type cultures of *P. capsici* and *P. tropicalis.* The isolates could be primarily differentiated based on the formation of unrestricted lesions with fimbriate margin (Fig. 5a) and restricted lesions with yellow halo (Fig. 5b) on leaves.

Based on the differential expression of symptoms the isolates were separated into two major groups -Group I (*"capsici*-like") showing fimbriate margin in black pepper and restricted lesion in pothos, while Group II (*"tropicalis"*) showing restricted lesion with yellow halo in black pepper and widely spreading lesions in pothos with few exceptions.



The infectivity of two isolates (05-06, Fig. 6a and 98-93, Fig. 6b) was compared with that of ATCC type cultures (ATCC 4034 and ATCC 76551) by SEM analysis. Interestingly, no hyphal penetration and ramification was observed in the case of both type cultures while the black pepper isolates (05-06 and 98-93) penetrated and ramified the pepper leaf tissues

#### Black pepper infected by P. tropicalis

Host range studies in comparison with type cultures of *P. capsici* (ATCC 4034) and *P. tropicalis* (ATCC 76651) indicated that in contrast with 05-06, ATCC 4034 produced hypersensitive reaction in black pepper but was avirulent to pothos (Fig. 6c). On the other hand, ATCC 76651 induced expanding lesions in pothos and produced restricted lesions with yellow halo in black pepper (Fig. 6d) as observed with 98-93. It was also evident that *Phytophthora* infecting black pepper comprises of more than one species, *P. tropicalis* as well as *P. capsici*-like species.







Fig. 6. Host range studies in comparison with type cultures of *P. capsici* and *P. tropicalis* 

#### Genes involved in *Piper colubrinum - Phytophthora capsici* interaction

Candidate genes known to play a role in defence signalling or in HR reaction were studied in *P. colubrinum* challenged with *P. capsici* using qPCR. The gene, chorismate synthase showed peak expression at 16 hpi. Genes like metacaspase, nitrate reductase and cinnamate 4 hydroxylase showed high expression at 4 hpi , 24 hpi and 72 hpi, respectively.

Screening of new molecules against *Phytophthora* All the six new fungicide molecules *viz.*, cymoxanil 8% + mancozeb 64% (Curzate 60DF, 100ppm-500ppm), iprovalicarb + propineb (Melody Duo, 750 ppm-1750 ppm), propineb (Antracol, 100 ppm – 500 ppm), chlorothalonil (Chlorothalonil 75WP, 500 ppm – 1500 ppm), famoxadone + cymoxanil .

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(Equation Pro, 200 ppm – 600 ppm) and Metalaxyl - mancozeb (Ridomil Gold, 62.5 ppm – 1000 ppm) evaluated in five different concentrations against *Phytophthora* showed 100% suppression of *Phytophthora* sp

#### Actinobacteria, a novel group of biocontrol agents

Out of the 50 actinobacterial strains tested, three *Streptomyces* isolates (IISRBPAct 1, IISRBPAct 25 and IISRBPAct 42) showed more than 90% inhibition of *P. capsici* and *Sclerotium rolfsii*. IISRBPAct 1 showed maximum growth promotion and inhibition (98.10%) of *S. rolfsii* while IISRBPAct 25 showed highest control of *P. capsici* (80.73%). IISRBPAct2 in combination with IISRBPAct5 was found effective in controlling *Radopholus similis* infection.

#### Slow decline disease Field evaluation of new pesticides

Multilocation trials on nematicidal activity of carbosulfan 6 G and flubendamide 20 WG indicated that both the pesticides effectively suppressed *R. similis.* 

#### Screening of botanicals

In vitro studies indicated that the leaf extracts of Simarouba glauca (Lakshmi taru) and Manihot esculenta (Cassava) effectively inhibited R. similis and M. incognita.

#### Anthracnose disease Evaluation of fungicides

Among the fungicides evaluated, initial spray with Bordeaux mixture (1%) at 30 DAP followed by fenamidone – mancozeb (0.2%) at 45 DAP was more effective in reducing the disease under nursery condition.

#### Viral diseases

Elimination of Piper yellow mottle virus (PYMoV) Cyclic somatic embryos, obtained from the micropylar region of mature seeds from PYMoV infected black pepper plants of six varieties *viz.*, IISR Malabar Excel, IISR Shakthi, IISR Thevam, Panniyur 1, Sreekara and Subhakara, were regenerated and hardened in greenhouse. PCR of 227 somatic embryo-derived plants showed that 65 plants (28%) were positive to PYMoV. Variety wise data indicated 55- 100% virus elimination in different varieties. A protocol for meristem tip culture of black pepper was developed. About 84% of the hardened plants, when tested through PCR were free of PYMoV. Complete elimination of PYMoV from black pepper could be achieved when somatic embryogenesis or meristem tip culture was combined with ribavirin, an antiviral agent.

### CARDAMOM Genetic resources

A total of 618 cardamom accessions are being maintained at National Active Germplasm Site. One accession was collected from Vallakadavu forest range, Periyar Tiger Reserve, Kerala.

Field screening of 106 cardamom accessions for leaf blight and rhizome rot resistance yielded five resistant accessions to rhizome rot (FGB 135, FGB 143, FGB 149, FGB 152 and FGB 159) and 36 resistant accessions to leaf blight.

#### Breeding

In Preliminary Evaluation Trial, the hybrid PV2 x IISR Vijetha registered more number of tillers, leaves, highest fresh and dry weight of the capsules.

#### Transcriptome analysis

PacBio single-molecule real-time sequencing in cardamom was done with RNA isolated from leaf samples of IISR- Vijetha plants challenged with cardamom virus. 56439 reads of insert with mean read length of 2267 bp in small cardamom was obtained with PacBio. The error corrected high-quality 8,351 isoforms were further clustered into 5253 contigs using Cap3 sequence assembly program.

A bicistronic transcript coding for palmitoyl-acyl carrier protein thioesterase and ent-kaur-16-ene synthase was discovered. A transcript coding for a photosystem II gene having inverted repeats region for the coding sequence was also identified.

#### **Crop Management**

Trials on nutrient management in cardamom indicated that the yield was significantly higher in the treatments involving FYM + neem cake (1.31 kg fresh plant<sup>-1</sup>) followed by FYM + vermicompost (1.17 kg fresh plant<sup>-1</sup>).

#### Plant Health Management

#### Viral disease

Kokkekandu (vein clearing) disease was noticed with high incidence (up to 60%) and severity only in Sirsi and Sakleshpur taluks of Uttara Kannada and Hassan districts of Karnataka, respectively.

#### Leaf blight

Natural incidence of leaf blight was recorded in 119 accessions and three released varieties (Appangala 1, IISR Avinash and IISR Vijetha). Among different morphotypes, maximum Per cent Disease Index (PDI) was recorded in Malabar (23.41-27.72), followed by Mysore (18.79-20.34) and Vazhukka (18.74-20.38).

## Virulence characterization of Colletotrichum gloeosporioides

Virulence analysis of 20 isolates of *C. gloeosporioides* employing molecular markers (RAPD, SSR and ISSR) revealed that only ISSR markers were linked with virulence of the pathogen.

#### Endophytic fungi

A pot culture experiment with shortlisted endophytes indicated that Cb2 (against *F. oxysporum*), AgR5D (against *P. vexans*), AgR5A (against *R. solani*) and Asupe 1 (against *C. gloeosporioides*) were promising.

#### Effect of promising isolate of Trichoderma

The effect of promising isolate of *Trichoderma* on growth inhibition of *Fusarium oxysporum*, *Rhizocto-nia solani* and *Pythium vexans* was also tested and growth inhibition against the pathogens was found to be maximum at higher concentration (20%) (Fig. 7).





#### Cardamom Thrips

#### Toxicity of insecticides to honey bees

Among the 4 promising insecticides (fipronil, 0.005%, imidacloprid 0.0089%, spinosad 0.0135% and quinalphos 0.05%) evaluated under laboratory conditions for their residual toxicity to honey bees, quinalphos was highly toxic and Spinosad was the least toxic to honey bees.

#### Evaluation of IPM strategies

Evaluation of IPM strategies for the control of cardamom thrips indicated that two sprays of spinosad 0.0135% and soil application of the entomopathogenic fungus, *Lecanicillium psalliotae* twice alternatively during March, April, May and August was effective in managing the pest.

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## **GINGER**

### Genetic resources

Presently, 668 accessions are being maintained in the field gene bank. The germplasm conservatory was enriched with 20 accessions collected from West Bengal, Nagaland, Kerala and Arunachal Pradesh. The special collections include extra bold ginger and red ginger from Arunachal Pradesh (Fig. 8).

#### Breeding

Among the eight accessions evaluated for high yield and low fibre content, Acc. 278 was found to be promising.

#### In vivo irradiation of ginger

IISR Rejatha was subjected to gamma irradiation at different doses of 0.8, 1.0 and 1.2 kR. All the M1V1 mutants were established in the green house for upscaling screening against Pythium sp. Besides this, 10 M1V4 and 102 (M1V9) mutants have been maintained. Three potential mutants 'resistant' to *Pythium* sp. were identified (V 0.5/2, R 0.8/1 and R 1.25/4) for further confirmation.

#### Generation of autotetraploids

The rhizome buds of IISR Rejatha were submerged in different concentrations of Colchicine (0.025, 0.050, 0.075, and 0.1 %) and Oryzaline (5, 10, 25, 50, 100  $\mu$ M) water solution for two different durations (24 h and 72 h) to induce polyploidy. Maximum sprouting was recorded in 0.025% colchicine and 5  $\mu$ M Oryzaline at 24 h.



Fig. 8. Red ginger accession collected from Arunachal Pradesh.

#### **Crop Management**

Fertigation in ginger using soilless medium consisting of coir compost and FYM in 1:1 proportion indicated that 75% of recommended dose of fertilizers applied through drip irrigation on daily basis produced maximum mean rhizome yield per plant (265g plant<sup>-1</sup>) followed by 100% recommended dose (254 g plant<sup>-1</sup>).

## Photosynthesis and partitioning of dry matter under coloured shade nets in ginger and turmeric

Photosynthetic rate was highest in plants grown under red shade net in ginger (5.3  $\mu$  moles m<sup>-2</sup> s<sup>-1</sup>) while it was highest in plants grown under open conditions in turmeric (5.8  $\mu$  moles m<sup>-1</sup> s<sup>-1</sup>). Dry matter partitioning to rhizomes was on par in plants grown under different shade nets in ginger while it was highest in plants grown under open conditions in turmeric, at 120 DAP. Rhizome yield was highest in plants grown under red shade net in ginger while it was highest in plants grown under open conditions in turmeric.

#### Plant Health Management

#### Foliar diseases

Surveys were conducted in ginger and turmeric growing areas of Kerala, Karnataka and Tamil Nadu and samples were collected from 49 locations. The isolated included Phyllosticta funai SD., Helminthosporium sp., Fusarium SDD., C. gloeosporioides, C. capsici, Exerohilum sp., Pestalotia sp. Curvularia hawaiinensis and a few unidentified funai.

#### Helminthosporium leaf blight

Leaf blight of ginger caused by *Helminthosporium* sp. was found to be severe in ginger growing tracts of Mysore, Chamrajnagar and Uttara Kannada districts of Karnataka and Wayanad and Kozhikode districts of Kerala.

#### Bacterial wilt

#### Cross infectivity of R. solanacearum strains

Cross infectivity studies on ten *R. solanacearum* isolates from different crops and geographical locations using three host differentials showed that all the sequenced isolates are infective on tomato and chilli whereas ginger was infected by only the ginger isolates.

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#### Defence responses

The interaction of *R. solanacearum* with resistant mango ginger and susceptible ginger plants was studied using nine candidate genes in different time course intervals by real-time PCR. The results showed that there was a strong and earlier expression of the selected transcripts in resistant mango ginger suggesting the involvement of these processes in the early containment of the pathogen.

#### Transcriptome-wide identification and characterization of resistant gene analogs

R-gene products obtained from the transcriptome of mango ginger and ginger were separated into distinct but related protein classes, according to their conserved structural domains. Screening for R-genes resulted in the identification of 160 clusters in mango ginger and 212 clusters in ginger with similarity to known R-genes. The gene expression studies of selected NBS-LRR transcripts identified that the R-genes were highly expressed in mango ginger in the initial hours post inoculation indicating their possible role in disease resistance.

Apoplastic bacterium for control of bacterial wilt Soil solarization coupled with seed treatment and soil application (at the time of planting and 30, 45 and 60 days intervals) with the apoplastic bacterium, *Bacillus licheniformis* (GAB 107) inhibited bacterial wilt, whereas under challenge inoculated conditions 81.73% inhibition was obtained.

#### Calcium chloride controls bacterial wilt

Solarization of soil followed by application of 3% calcium chloride (soil application at the time of planting and thereafter at 30, 45 and 60 days intervals) resulted in 100% inhibition of bacterial wilt in sick field and 98.21% inhibition under challenge inoculated conditions.

#### Secondary metabolites of Bacillus licheniformis

The secondary metabolites of *B. licheniformis* (GAB 107), extracted using ethyl acetate caused >90% inhibition to *R. solanacearum* and >75% inhibition to *P. myriotylum*, the causal agents of bacterial wilt and soft rot of ginger, respectively. Volatiles of *B. licheniformis* are also inhibitory to *R. solancearum*.

#### Viral disease

Surveys were conducted in ginger fields of Karnataka (Chamarajanagar, Mysore, Uttara Kannada, Kodagu and Hassan districts) and Kerala (Wayanad, Idukki, Kozhikode districts) and the incidence of viral disease ranged from 10-70%.

## Shoot borer

#### Molecular characterization

*Conogethes punctiferalis* populations infesting cardamom, ginger, turmeric and *Ammomum* sp. collected from nine locations in Kozhikode, Idukki and Wayanad Districts of Kerala, Kodagu District in Karnataka and Dimapur District in Nagaland were characterized using universal primers.

#### Association of Wolbachia sp.

The association of *Wolbachia* with *C. punctiferalis* populations infesting ginger, turmeric and cardamom was confirmed through multi-locus sequence typing (MLST). The populations were found to be super infected by *Wolbachia* super groups A & B.

#### Seasonal incidence in relation to crop phenology

The incidence of shoot borer infesting ginger and turmeric in relation to crop phenology was studied at fortnightly intervals. On ginger, the shoot borer infestation was first observed during the second fortnight of August and was high during the second fortnight of October. On turmeric, the pest infestation was first observed during the second fortnight of July and was high during the first fortnight of November.

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#### Screening of insecticides

Screening of 10 insecticides for their bioefficacy against shoot borer was carried out for the second consecutive year in ginger and turmeric under field conditions. On ginger, plots treated with chlorantraniliprole 0.01% had minimum pest infestation on the shoots that was on par with flubendiamide 0.02%, spinosad 0.135% and cyantraniliprole 0.005%. Chlorantraniliprole 0.01% treated plots recorded minimum pest infestation in turmeric that was on par with lamda-cyhalothrin 0.0125%, fluben-0.02%. fipronil 0.003% diamide and cyantraniliprole 0.005%.

#### Field evaluation of EPNs

Infectivity of promising EPNs such as *Steinernema* sp. (IISR-EPN 02) and *Oscheius gingeri* (IISR-EPN 07) was tested against shoot borer *Conogethes punctiferalis* infesting ginger and turmeric under field conditions. Among the treatments, integrated treatment with IISR-EPN 02 and malathion showed less shoot damage in ginger and turmeric (12.6 and 20.8%, respectively) which was on par with the malathion (14.7 and 22.6 %, respectively). In another trial, conducted in a farmer's plot, integrated treatment (IISR-EPN 07 + malathion and IISR-EPN 02 + malathion) showed less shoot (5.5 and 5.7%, respectively) compared to 21% in control.

#### Other insect pests Infectivity of EPNs

Among the six entomopathogenic nematodes tested under *in vitro* conditions against *Spodoptera* sp. and root grub *Leucopholis coneophora* Burm infecting ginger, *Steinernema* sp. (IISR-EPN 02) and *O. gingeri* (IISR-EPN 07) caused 100% mortal-ity within 72 h.

### TURMERIC Genetic resources

To date, 1404 Curcuma accessions are being maintained in the field gene bank. The germplasm conservatory was enriched with 69 turmeric germplasm received from four centres.

Characterization of 102 turmeric accessions was done for different morphological traits as per the DUS guidelines. Among the qualitative characters, pseudostem growth habit and leaf disposition exhibited maximum variability. Maximum yield per plant was recorded in Acc. 48 with bold fresh rhizomes. Acc. 849 was unique with elongated mother rhizome and has purple pigmentation in the leaf mid-rib.

Forty four RAPD primers and 31 microsatellite (SSR) primers were used for molecular profiling of 10 genotypes. The dendrogram generated from the data revealed that the selected genotypes are distinct from each other. Accession 849 was found to be distinct from all the other genotypes in accordance with its distinct morphological identity.

#### Breeding

A high yielding, short duration turmeric variety has been developed through germplasm selection. The variety, named IISR Pragati (Fig. 9), was tested as Acc.48, for over three years in different turmeric growing regions of the country and under various climatic conditions. The characteristic features are



# Surveillance and documentation of pests and diseases

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Forty three plantations of cardamom (9), black pepper (19) and cardamom ~ black pepper mixed cropping systems (15) representing diverse agro-ecological regions were surveyed for the occurrence of pests and diseases. In black pepper, foliar infection/foot rot due to *Phytophthora*, slow decline, anthracnose/spike shedding/necrosis and blight due to *Colletotrichum*, stunt disease were the major diseases. Foliar blight, an atypical symptom induced by *Colletotrichum* was more pronounced during post-monsoon period. Infestation of pollu beetle and scale insects were the major pests observed during the surveys.

In cardamom, leaf blight incited by *Phytophthora* and *Colletotrichum*, stem lodging and capsule anthracnose were the major diseases. The major viral diseases recorded during the survey were chlorotic streak, kokkekandu and katte. Pseudostem borer, thrips and infestation of minor pests were also observed in the surveyed regions.

#### Documentation of natural enemies of spice crop pests

Surveys for incidence of natural enemies of spice crop (black pepper, cardamom, ginger, turmeric, nutmeg, allspice and clove) pests were conducted in 34 locations in Kerala, Karnataka and Tamil Nadu. Three entomopathogens were documented from *Sinoxylon* spp., *Marsipococcus marsupialis* and *Mimegralla coeruleifrons*. The fungus infecting Sinoxylon spp. was identified as *B. bassiana* (IISR-EPF-13) and that infecting *M. marsupialis* as Isaria sp. (IISR-EPF-14). An entomopthoralean fungus recorded from *M. coeruleifrons* has been tentatively identified as *Batkoa sp*. (IISR-EPF-15).

## High Value Compounds and Phytochemicals

## Antioxidant activity of spice extracts

Combinations of spice extracts (cinnamon: turmeric (2:1) showed maximum antioxidant potential tested by DPPH scavenging activity

## In vivo anti inflammatory potential of turmeric and cinnamon

Study conducted in normal rats administered with methanolic extract of cinnamon and turmeric (2:1) revealed that the activity of the enzymes catalase (CAT), Superoxide Dismutase (SOD), Glutathione-Stransferase (GST), Lacatate Dehydrogenase (LDH), Malate Dehydrogenase (MDH), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were not affected indicating no adverse effect of the extract.

#### In vitro cytotoxicity

Studies on the in vitro cytotoxic effect of spice extracts carried out at RCC Thiruvananthapuram on the cell lines SKBR-3 (breast cancer) and HCT-116 (colon cancer) indicated that all extracts possessed concentration dependant activity between 50  $\mu$ g ml<sup>-1</sup> and 800  $\mu$ g ml<sup>-1</sup> and hexane extract of cinnamon had the highest cytotoxic effect.

## Hypoglycemic potential of black pepper, cinnamon and turmeric extracts

In vitro hypoglycemic potential of methanol extract of cinnamon, turmeric and cinnamon: turmeric mixtures (2:1, 1:1 ratios) were studied using  $\alpha$  amylase enzyme. Higher inhibitory potential was observed for cinnamon extract followed by cinnamon: turmeric mixture (2:1 ratio).  $\alpha$ - glycosidase inhibitory assay also showed the same trend.

#### Study on spice crops in Darjeeling, West Bengal

Institute undertook a meticulous study of the major technical constraints in enhancing production and productivity of large cardamom, ginger and turmeric in Darjeeling district of West Bengal. The initiative guided by the Horticulture Sciences Division of ICAR was undertaken in collaboration with Spices Board, Uttar Banga Krishi Visva Vidyalaya, Pundibari and Department of Agriculture, Government of West Bengal and other institutional stakeholders. The study team brought out the important extant technical constraints in the selected crops and submitted a detailed report containing the recommendations and a multipronged action plan for enhancing productivity of these crops through technology infusion, adaptive research programme and value chain support activities.

## **Spices Economics**

#### Growth, instability and export trends in small cardamom

The instability of key variables affecting small cardamom production in the country during the last four decades was analyzed critically to explore the patterns of instability. A marked decline in instability was identified in production, yield and export quantity. The export quantity of small cardamom showed a higher compound annual growth rate (3.53%) during the second period (1993-94 to 2014-15) accompanied by a sharp decline in instability by 52.7% in comparison to the first period (1971-72 to 1992-93). The decline in yield and production instability between the two periods was 44 and 33%, respectively.

The share of domestic production exported declined from 46.3% for the triennium ending 1974-75 to 20.0% for the triennium ending 2014-15. Though the production of small cardamom increased by 2.5 times during the last three decades, the share of domestic production in exports of cardamom remained constant. India's share in global cardamom exports have also declined from 48.6% in 1974-75 to 10.1% in 2014-15.

#### Analysis of price instability in spices

A study was conducted on the price movement of important spice crops to understand the price fluctuations in major spices and their magnitude. Using monthly time series nominal price data of spice commodities, the real prices were constructed using WPI (2004-05 series) index as deflator. A fluctuation margin for prices was considered at 20% deviation from predicted prices constructed using normal forecasting techniques

## Relative contribution of yield growth to spices production

A simple decomposition model was used to measure the relative contribution of yield component to increase in production of spices. The results indicated that the yield enhancement has contributed significantly to the increase in spice output. The results were similar across several spice crops like cardamom, turmeric and ginger where the direct and indirect yield effects contributed more than 50% of increase in output. For spices production as a whole the area effect, yield effect and interaction effect were 32.4, 45.2 and 22.4%, respectively.

## INSTITUTE TECHNOLOGY MANAGEMENT AND BUSINESS PROCESS AND DEVELOPEMINT

During the year, the ITM-BPD Unit has issued three licenses for black pepper varieties IISR Thevam and IISR Girimunda to two clients. Turmeric variety, IISR Alleppey Supreme and ginger Variety IISR Varada were licensed to Centre for Overall Development, Thamarasserry, Kerala. The new turmeric variety IISR Pragati is under consideration for registration by PPV and FRA. Two clients have availed the license for commercial multiplication of IISR Pragati.

The Unit organized and conducted the market launch programme on 27.06.16 for two products "Powercap" and "Trichocap" on behalf of the licensee, M/s Codagu Agritech, Karnataka in presence of Honorable MP, Mysuru, Mr. Pratap Simha.



A planting material production unit cum nursery for spices, ornamentals, plantation and fruit crops was established at IISR, Chelavoor. License agreement for cleaning, grading, packing and powdering of black pepper and white pepper was signed with CAMPCO Pvt India Ltd. on 23rd February at Mangaluru (Fig. 12b).



Fig. 12b. Signing of MoU with CAMPCO Pvt India Ltd

The unit conducted two EDP programmes, one on curry powder production during July 2016 and another on fruit and vegetable processing for women self help groups during August 2016. The unit also participated in VAIGA-2016, exhibition organized during the "International Workshop on Agro Processing & Value Addition" conducted by Department of Agriculture, Development & Farmers' Welfare, Government of Kerala. The unit also participated in Kisan Melas and exhibitions for showcasing commercialized technologies of IISR.

## EXTENSION AND TRAINING

The transfer of technology programmes of the institute was provided through regular channels like visitor advisory services which provided specific advice to more than 2700 farmers. The other visitors included 1800 students and 250 officials.

Skill development and capacity building were implemented under the sponsorship of various state departments and central sector schemes of Tribal Sub Plan and programme for NE region. Thirteen training programmes were conducted on campus benefiting more than 300 participants including stakeholder groups like farmers, youth, tribal beneficiaries and students. Under the Tribal sub plan eight training programmes were organized off campus benefiting 600 tribal farmers from the states of Kerala, Tripura and Arunachal Pradesh. The institute also facilitated the monthly technology advisory meeting of the district Agricultural Technology Management Agency (ATMA) by providing expert support for resolving field problems. The institute was also featured in a special educational programme as leading destination for higher education in the field of agricultural research. Eighteen programmes benefiting more than 100 farmers were organized.

A total of 48 exhibitions days were organized by the institute during the last year. The institute facilitated a total of 50 group visits for educational institutions to provide exposure to research and development activities in spice crops. About 15 farmer groups from within and outside the state under MIDH or ATMA visited the institute for learning about the technologies developed for improved spices productivity.

Several demonstrations aimed at tribal farmer empowerment were undertaken. Varietal demonstrations in black pepper were under taken in Kerala, Tripura and Arunachal Pradesh. Demonstration in collaboration with the Spices Board, on application of *Trichoderma* in capsule forms in black pepper and ginger is in progress in four districts of Arunachal Pradesh and in Shillong and Guwahati. Demonstration of single sprout transplanting method in ginger was laid out in four KVKs in Tripuara.

In tune with the policy objective to step up revenue generation, efforts were taken through ATIC to generate more income from the sale of technology inputs and services which resulted in an increase of 143 % in revenue compared to last year.

#### Varietal dissemination

Feedbacks from farmers' plot revealed very good acceptance of the released turmeric and ginger varieties. Mr. Ramaprasad Reddy of Hyderbad, an IT professional turned turmeric grower, who has been growing Prathibha for the the last three years has ventured into value addition of turmeric. Mr. V. Sureskumar of Kalarkode, Alleppey is another small scale entrepreneur who in a vlaue chain mode market powdered Prathibha in southern Kerala.



Fig. 13. Mr. Jojo Jacob with his harvested Varada

Mr. K.C. Joseph Inchakkal, a small farmer from Venappara on the eastern part of Kozhikode, Kerala who grew Varada ginger in about 2.5 acres (intercrop of rubber plantations) reported about 40 q production this year. Mr Jojo Jacob, an ICAR-IISR KVK trained youth of Kadiyangad, Kozhikode district too obtained very good yield from Varada ginger (Fig. 13). Mrs. Elzy Devasia, a women farmer from Chempanoda, Kozhikode harvested on an average 1.5 kg fresh rhizome from Varada riaised in grow bags.

#### Integrated black pepper research and development in North Kerala districts

Twenty four FLDs on improved technologies that were initiated at farmer's plots in three panchayaths of Kozhikode district during last year were continued with necessary input supply along with two participatory nurseries (Fig. 14). Five visits were made by the scientists to the FLD plots for giving advisories.

Six hundred and twenty soil samples from farmer's plots were analysed for macro and micro nutrients and issued with soil health card advisories. Demonstrations on site specific nutrient management were taken up in five farmer's plots. Amendments and fertilizer doses were applied based on the soil test and foliar supplementation of micronutrient based on leaf nutrient ratios were taken up.



Fig. 14. Technology demonstration: Black pepper variety – Subhakara at Mr. Agustin, Chakkittupara

Due to site specific management, the soil pH, OC, P, K, Ca, Mg and micronutrient availability showed a significant increase as compared to farmers practice. The spike intensity and yield (std-1) also showed a significant increase in soil application of fertilizers (29.30.5 m<sup>-2</sup> & 1.45 kg dry std<sup>-1</sup>) and fertilizers + micronutrient foliar supplementation (30.50.5 m<sup>-2</sup> & 1.48 kg dry std<sup>-1</sup>) treatments as compared to farmers practice (25/0.5 m<sup>-2</sup> & 1.3 kg dry std<sup>-1</sup>). The site specific nutrient management also helped in increasing the bulk density (448 g/L<sup>-1</sup> in farmers practice and 500 g L<sup>-1</sup> in treatments) and piperine content (5.3% in farmers practice and 5.6-6.5% in treatments).

#### Enhancing the economic viability of coconut based land use systems

In pepper, application of amendments (dolomite + gypsum) significantly improved the pH of the surface (0-15 cm) and sub surface soil (15-30 cm) as compared to farmers practice. The soil availability of P, K, Ca, Mg and Zn also increased significantly at both the depths studied as compared to farmer's practice. The adoption of site specific soil fertility management resulted in significantly higher yield (1.6-1.78 kg dry std<sup>-1</sup>) as compared to farmers practice (0.98 kg dry std<sup>-1</sup>). In demonstration plots 33-68% increase in yield was recorded under site specific management treatments in addition to increase in oleoresin, piperine contents and bulk density as compared to farmers practice.

Under nutmeg system also the soil pH increase was higher in lime + gypsum treatment on par with that of lime application alone at both the soil depths studied. Lime + gypsum supplementation with site specific fertilizer application recorded 30% higher nut and mace yield (3.9 & 0.6 kg fresh tree<sup>-1</sup>) as against farmers practice (3.0 & 0.45 kg fresh tree<sup>-1</sup>). In demonstration plots an increase of 18-28% in fruit yield was observed in treated trees over farmers practice.



Fig. 15. Awareness campaign for farmers and extension functionaries to demonstration plots under the project

#### Area wide integrated pest management

Three demonstration plots were laid out in Muthappanpuzha (Kozhikode District, Kerala) for demonstrating the different packages for management of foot rot and slow decline disease of black pepper. Three black pepper nurseries were established in Omasserry, Muthappanpuzha and Anakkampoyil for demonstrating production of healthy and disease free planting materials in farmers' plots in a participatory mode. Biocontrol agents *T. harzianum* + *P. chalmydosporia* followed by *Ketasatospora setae* + *Streptomyces* tauricus were found promising in producing disease-free and healthy planting materials.

## KRISHI VIGYAN KENDRA

During the period, KVK conducted total of 112 on-campus and off-campus training programmes. A total of 4153 trainees were benefitted. Two on Job Trainings to Vocational Higher Secondary School students (89 numbers) were organized. A seven day's training programme on soil testing was conducted for six students of AVAH Arts and Science College, Meppayyur. Three day's paid training programmes on broiler goat rearing; breeding and culture of fresh water ornamental fishes and bee keeping were organized. National Fisheries Development Board (NFDB), Hyderabad sponsored five and three day's duration trainings on "Fresh Water Ornamental fish culture" and "Fish



Fig. 16. Hon'ble Union Minister Shri. Radha Mohan Singh presenting the Deen Dayal Upadhyaya Anthyodaya Agriculture Award 2016

Processing Techniques" were conducted for 60 participants. Twenty five farmers of Kozhikode district were trained on "Cocoa cultivation and value addition" under the training sponsored by Directorate of Cocoa and Cashew Development, Cochin. In addition, three inter institutional collaborative training programmes on "Scientific coconut cultivation and value added products development" with CPCR1, Kasaragod; "Tapioca based biological pest management in Banana" with CTCRI, Trivandrum and "Spices propagation and integrated management" with IISR, Kozhikode were also organized.

A mass awareness programmes and presentation ceremony of the "Pandit Deen Dayal Udpathya Anthyodaya Agriculture Award 2016" was organized at ICAR-IISR, Kozhikode on 25th September 2016. The award was presented Hon'ble Union Minister for Agriculture and Farmers Welfare Shri. Radha Mohan Singh to Shri. Baburaj, an aqua farmer from Kozhikode, Kerala.



Fig. 17. Farmers fair and awareness programme on "Pradhan Mantri Fasal Beema Yojana" inauguration by Sri. M.K. Raghavan, Honourable Member of Parliament (Lok Sabha)

Eight Front Line Demonstrations and seven On Farm Trials on technology assessment and demonstration in 146 farmers' fields were carried out.

Seminar cum training on "Scientific coconut cultivation"; farmers fair and awareness programme on "Pradhan Mantri Fasal Beema Yojana": celebration of Agriculture education day; World soil day and Rabi awareness programme on "Organic vegetable cultivation"; Jai Kisan Jai Vigyan Week - 2016 and "Agriculture Farmers' Innovation Meet" were organized at KVK, Peruvannamuzhi. The Kendra conducted two field days, four seminars, participated in 12 exhibitions, delivered seven radio talks and conducted three studies cum exposure tours for farmers to various research institutes. During the period, KVK organized about 10 awareness programmes on soil health management, and issued 225 soil health cards. The KVK also involved in production of quality planting material of black pepper and nutmeg.



Fig. 18. Shri. V.S. Sunilkumar, Hon'ble Minister for Agriculture, Govt. of Kerala visiting KVK stall at Kootalida, Kerala



Fig. 19. Rabi awareness programme on "Organic vegetable cultivation" - lecture delivered by Dr. P Raji, RARS, KAU, Pattambi

## ICAR-ALL INDIA COORDINATED RESEARCH PROJECT ON SPICES

In the XXVII work shop of AICRP on Spices held at ICAR-NRC on Seed Spices, Ajmer, during 24-26 October 2016, seven new varieties were recommended for release.

Six location specific technologies on spices for various states were recommended

Application of Ponneem (2 ml l<sup>-1</sup>. of water) was recommended for controlling shoot and capsule borer in cardamom for Kerala.

Pre-planting treatment of seed rhizome and foliar spray of standing crop at 90, 105, 120 days after planting with propiconazole (0.1%) was recommended to control foliar disease of turmeric in Bihar.

Seed rhizome treatment with carbendazim + mancozeb (1:1) (0.1%) + foliar spray of carbendazim + mancozeb (0.1%) on 45 and 90 days or seed rhizome treatment with azystrobin (0.1%) + spray on 45, 75 and 105 DAP for the management of *Colletotrichum* and *Taphrina* leaf blotch of turmeric for Chhattisgarh.

## HUMAN RESOURCES DEVELOPMENT

Hexaconazole spray two times at 20 days interval (0.1%) was recommended for management of *Phyllos-ticta* leaf spot in ginger in Himachal Pradesh.

Carbendazim (0.1%) + mancozeb (0.1%) first at disease appearance and subsequently two sprays at 20 DI after 1st spray or foliar spray with propiconazole (0.1%) first at disease appearance and then two times at 20 DI or foliar spray with tricyclazole (0.1%) first at disease appearance and then two times at 20 DI was recommended for Bihar.

Foliar spray with carbendazim:mancozeb (1:1) (0.1%) first at a disease appearance and then two times at 20 days interval was recommended for Chhattisgarh.

Hexaconazole (0.1%) or Propiconazole (0.1%) spray first at disease appearance and then two times at 20 days interval was recommended for Pundibari, West Bengal.

The Bioinformatics Centre (DISC) organized a short-term training "Bioinformatics for Transcriptome Analysis" during 22-25 March 2017. Sixteen participants from various ICAR institutes, SAUs and other institutions participated in this four day program. Apart from the Institute faculty, experts from Central University of Kerala, Xcelris Labs Ltd., Ahmedabad and NCBS, Bengaluru handled various classes.

Conducted 10 days in-plant training programme for students at KAU, Tavanur during 1-10th March 2017



Fig. 20. Faculty members and trainees of short-term training on bioinformatics for Transcriptome Analysis

### **MAJOR EVENTS OF ICAR-IISR**

Event	Particulars
Swachhta Pakhwara celebrations	17-28, May 2016
World Environment Day	5 June 2016
International Day of Yoga	21 June 2016
Presentation ceremony of the "Pandit Deen Dayal Udpathya Anthyodaya Agriculture Award 2016	25 September 2016
Interactive meeting of ICAR-IISR scientists with UPASI Spice Committee members	10 August 2016
Swachhta Pakhwara celebrations	17-31, October 2016
Vigilance Awareness Week	31 October – 5 November 2016
Student-Scientist interface on "New Vistas in Invertebrate Research"	25th January 2017
Productivity Week	12-18 February 2017
Two days District Level Seminar on Spices	18-19, February 2017
National Science Day	25 February 2017

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Fig. 21. Dr. Rashid Pervez (Principal Scientist and Hindi officer) receiving the Best Official Language Magazine Award and Baibhasha Shield Award

### AWARDS

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### ICAR- Ganesh Shankar Vidyarthi Best Official Language Magazine Award

This award was granted for official language magazine Masloon Ki Mehak by Indian Council of Agricultural research, New Delhi at Vigyan Bhawan, New Delhi on 16 July 2016.

#### Rajbhasha Shield Award

This award was granted for significant contribution to Hindi correspondence, organizing Hindi workshops, OLIC meetings, publication like annual report, masala samachar, research highlights, official language magazine Masloon Ki Mehak, popular articles and extension bulletin in Hindi and OL implementation during 2015-16



#### ICAR-Indian Institute of Spices Research Marikunnu P. O., Kozhikode - 673012 Kerala, India. Phone: 0495-2731410, Fax: 0495-2731187 E-mail: mail@spices.res.in, Web site: www.spices.res.in