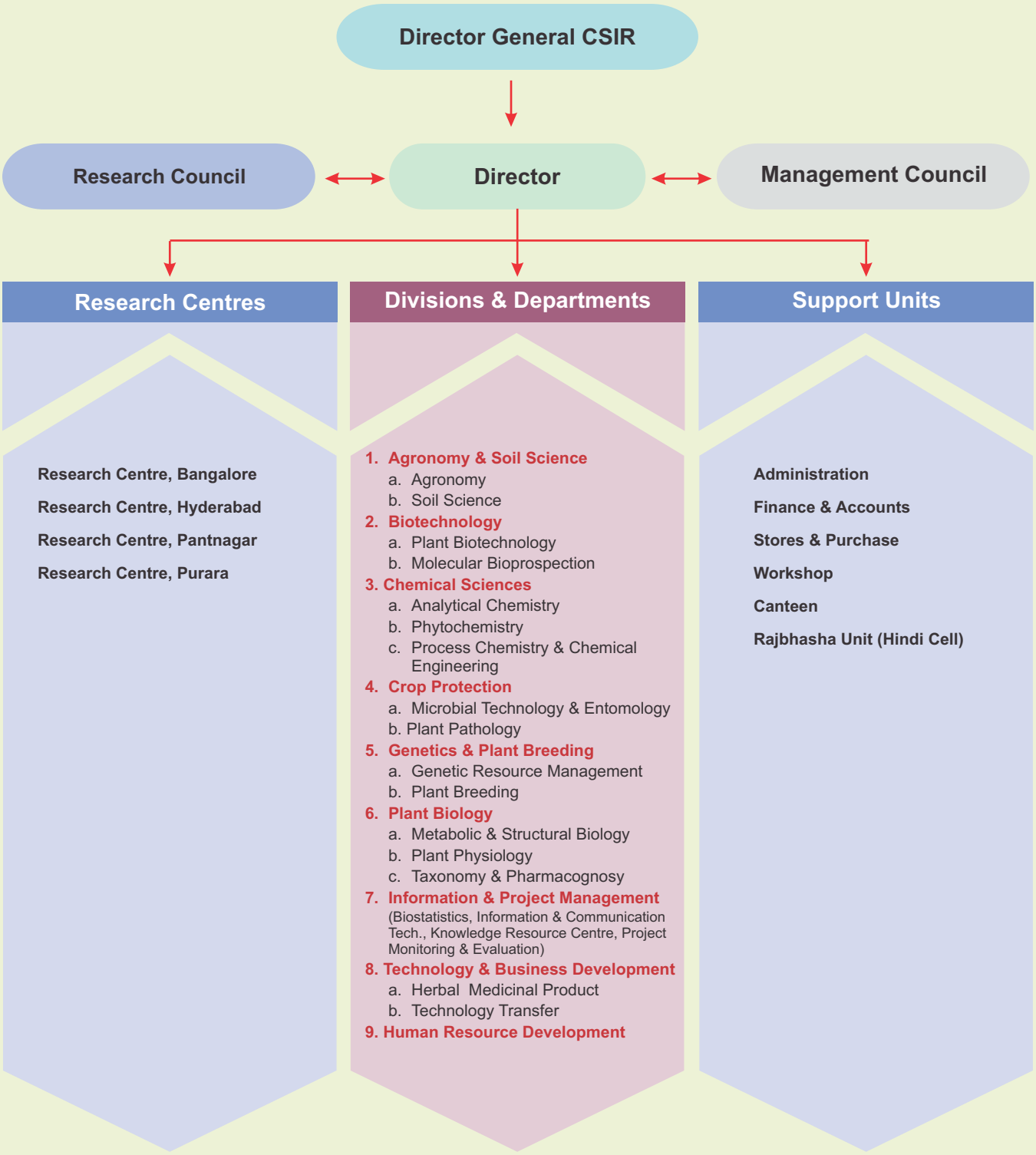
वार्षिक प्रतिवेदन
Annual Report
2010-2011

CSIR-CIMAP

CSIR – Central Institute of Medicinal and Aromatic Plants
(Council of Scientific and Industrial Research)
Lucknow | India

Organizational Structure



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Annual Report
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With Best Compliments from

Prof. Ram Rajasekharan
Director, CSIR-CIMAP

CSIR-CIMAP

CSIR – Central Institute of Medicinal and Aromatic Plants
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Scientists for providing timely inputs

A Team CSIR-CIMAP Effort

Contents

निदेशक की कलम से .....

समय और ज्वार के किसी के इन्तजार न करने वाले पुरातन मुहावरे को चरितार्थ करते हुए मेरे लिए अपने संस्थान के एक और वार्षिक प्रतिवेदन का आमुख लिखने का अवसर आ गया है।

इस वर्ष औषधीय एवं सगन्ध पौधों के विभिन्न शोध पहलुओं के अतिरिक्त मौलिक अनुसंधान की दिशा में भी सी.एस.आई.आर.-सीमैप ने सार्थक प्रगति की है। इस वार्षिक प्रगति को उच्च उत्पादन वाली नवविकसित पौध किस्में सीमैप-अजय (पोस्त-अधिक मार्फीन) सीमैप-मधु (स्टीविया-अधिक स्टीवियोसाइड़) और सीमैप-पात्रा (पिपरमिंट-अधिक मेन्थाफ्यूरान) द्वीप्तमान कर रही है। कृषकों का आर्थिक उन्नयन संस्थान की प्रमुख प्राथमिकताओं में एक होने के परिणामतः नवीन उच्च उत्पादकता वाली किस्मों का विकास संस्थान की महत्वपूर्ण उपलब्धि एवं कृषकों के लिए वरदान है।

इस वर्ष आगामी पंचवर्षीय योजना में सम्मिलित करने हेतु नई परियोजनाओं के निर्धारण में पर्याप्त समय एवं ऊर्जा विस्तृत वैज्ञानिक विवेचना के रूप में लगाया गया, जिसमें संस्थान के सभी वैज्ञानिकों ने भाग लिया और सर्वसम्मति से एक संयुक्त एवं नेटवर्क परियोजना (सुप्रा इंस्टीट्यूशनल परियोजना) का प्रस्ताव बना।

फरवरी 2011 में संस्थान को अन्तर्राष्ट्रीय बागवानी समिति द्वारा प्रायोजित अन्तर्राष्ट्रीय सम्मेलन ''पैपावर'' का आयोजन करने का अवसर मिला जिसमें अन्तर्राष्ट्रीय एवं राष्ट्रीय प्रतिनिधियों ने अच्छी भागीदारी की। इस सम्मेलन में पोस्तजन्य मादक एवं अमादक क्षाराभों के वर्तमान औषधीय एवं विवादग्रस्त प्रयोगों के बारे में विस्तृत चर्चा की गयी। मलेशिया सरकार के इन्नोवेशन केन्द्र यूनिक के साथ समझौते के करार-पत्र पर हस्ताक्षर कर केन्द्रीय औषधीय एवं सगन्ध पौधा संस्थान ने अपनी उपलब्धियों में एक और अनोखी वृद्धि कर ली। औषधीय एवं सगन्ध पौधों से सम्बन्धित ज्ञान भण्डार, तकनीकी जानकारियां अब तक केवल संस्थान की अनुकरणीय धरोहर थी पर अब उसे सीमा पार विस्तार आपसी सहमति के आधार पर मिलेगा।

आर्थिक मोर्चे पर भी संस्थान ने इस वर्ष असाधारण प्रदर्शन किया। नये वैज्ञानिकों की भर्ती एवं शोधार्थी छात्रों के अत्यधिक आवेदन संस्थान के वैज्ञानिक सामर्थ्य को परिलक्षित करते हैं।

मैं इस क्रम के सतत् बने रहने की आशा के साथ आने वाले वर्षों में इसके उत्तरोत्तर विकास की उम्मीद एवं इच्छा करता हूँ। मैं संस्थान के समस्त वैज्ञानिकों एवं कर्मचारियों का धन्यवाद् करते हुए यह कामना करता हूँ कि भविष्य में वे इसी लगन एवं उत्साह से कार्यरत रहेंगे।

राम

(राम राजसेखरन)

From the Director's Desk.....

As the age old proverb goes, “Time and tide wait for none”, it is already another year and it is time for me to give a foreword to yet another annual report of our Institute.

This year CSIR-CIMAP has made significant progress in some frontier areas of MAPs alongside the basic research breakthroughs. Some of the highlights from this year's progress include the release of a new opium variety CIMAP-Ajay, a high stevioside yielding stevia variety, CIMAP-Madhu and a high menthofuran yielding mint variety – CIMAP-Patra. This progress is very important for us, especially because we, as an institute strive towards the upliftment of farmers and newer high yielding varieties are boons to the farmers.

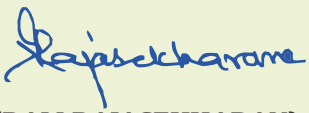
Considerable amount of time was also spent by all the scientists towards proposing new programs for the next five year plan. Extensive deliberations on proposing Supra Institutional Project and other Network Projects for the XII five year plan were conducted, wherein all the scientists of the Institute participated and brainstormed to come together and finally evolve a combined project proposal.

CSIR-CIMAP also had the opportunity to host the International conference on Papaver in February, which was well attended by National and International delegates. Current trends in the use and research of the economically important narcotic plant were discussed and the non-narcotic alkaloids of the plant were deliberated upon.

Adding a new feather to the cap, CSIR-CIMAP made a mark in the international arena by signing a Memorandum of Understanding with UNIK, an Innovation center of the Government of Malaysia. By this, the fund of knowledge, the know-how and technologies of growing medicinal and aromatic plants, which has this far been the inimitable property of CSIR-CIMAP will cross borders on mutually agreed terms.

CSIR-CIMAP did extraordinarily well with finance this year. Fresh recruitments of scientists and a bee line of students to join us for their PhD programs only go to prove the scientific prowess of the Institute.

I hope and wish that the trend continues and we, as an institute are able to perform better than this with every passing year. I extend my thanks to all my scientists, students and staff members and wish them good luck to continue with the same zeal and enthusiasm in the years to come.



(RAM RAJASEKHARAN)

CSIR-CIMAP

1. AGRONOMY & SOIL SCIENCE

Project Title: Enabling high value agriculture in low value under utilized soils and cropping systems

1.1. Influence of vermicompost as source of organic chelate on growth and yield of chamomile (*Matricaria recuitita*) in Ni, Cd and Cr polluted soils
(D.D. Patra, Mohd. Anwar, Sukhmal Chand)

Results of greenhouse experiment conducted during December 2009 to April 2010 revealed that out of three heavy metals (Ni, Cr, Cd) applied for simulation, Cr was found to be highly toxic to the plant at 20 ppm. On the other hand Ni and Cd at 20 ppm significantly enhanced the dry matter and flower yield of chamomile. Addition of vermicompost as the source of organic chelate enhanced the chelate mediated heavy metal accumulation by the crop. Although a sizeable amount of heavy metals was being translocated to roots, shoots and flowers, the blue oil extracted through hydro-distillation of flowers did not contain any heavy metal. Further, the quality of the oil in terms of chemical constituents was within the range of that obtained from chamomile grown under normal soil conditions.

Influence of vermicompost on accumulation of micronutrients in chamomile flowers under heavy metal enriched soils.

Treatments	Micronutrients uptake (mg g^{-1})			
	Fe	Cu	Mn	Zn
T_0	0.30	0.19	0.08	0.26
T_1	1.70	0.63	0.27	0.80
T_2	1.11	0.32	0.21	0.62
T_3	-	-	-	-
T_4	1.02	0.22	0.16	0.64
T_5	1.20	0.38	0.28	0.80
T_6	-	-	-	-
T_7	1.73	1.54	0.23	1.10
SE±	0.45	0.31	0.04	0.29
CD 5%	1.07	0.74	0.09	0.68

T_3 & T_6 plants did not survive

T_0 = Control, T_1= Vermicompost @ 2.5 g kg^{-1}, T_2= Ni @ 20 mg kg^{-1}, T_3= Cr@ 20 mg kg^{-1}, T_4=Cd @20 mg kg^{-1}, T_5= Ni @ 20 mg kg^{-1}, + Vermicompost@ 2.5 g kg^{-1} soil, T_6= Cr @ 20 mg kg^{-1}+ Vermicompost @ 2.5 g kg^{-1} soil, and T_7 = Cd @ 20 mg kg^{-1}, + Vermicompost @ 2.5 g kg^{-1} soil.

1.2. Influence of secondary plant nutrients (Ca, Mg) on growth and yield of chamomile (*Matricaria recuitita*) (D.D.Patra, R.K.Upadhyay)

A greenhouse experiment was conducted to study the influence of different levels of Ca and Mg on growth, yield and quality of *Matricaria*. The treatment comprised five levels each of Ca and Mg (0, 50, 100, 150 and 200 mg/ 10 kg soil) and their combinations. Effect of Mg was found to be more pronounced as compared to calcium in respect of plant height, number of branches per plants, flower size, number of flowers per plant and oil content in flowers. The interaction effect of Ca and Mg was maximum when both were applied at 200 mg/ 10kg soil. Both the secondary plant nutrients were responsible for improving the quality of the oil.

1.3. Influence of leather industry effluent mediated heavy metal polluted sludge on growth, yield and heavy metal accumulation by *Matricaria*
(Mohd. Anwar, Sukhmal Chand and D.D. Patra)

A pot experiment was conducted to elucidate the performance of *Matricaria* in heavy metal polluted conditions where sludges were added to soil in different ratios i.e., 4:0, 3:1, 2:2, 1:3 and 0:4. Results indicate that sludge alone resulted in high biomass, oil yield and heavy metal accumulation.

1.4. Integrated nutrient management in Indian basil
(Kambod Singh, R.S.Tripathi, Mohd. Yaseen)

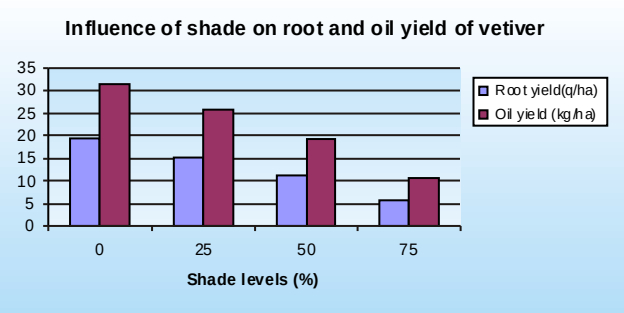
A field experiment was conducted to work out the nutrient requirement of *Ocimum basilicum* through integrated nutrient management approach for sustaining higher productivity and improving soil fertility. The treatments, consisting of ten different combinations of organic and inorganic fertilizers (Control, Full N:P:K100:40:40 kg ha^{-1}, Full FYM, Full Vermicompost, 75% NPK+25% FYM, 50% NPK+50%FYM, 25% NPK+75% FYM, 75% NPK+25% Vermicompost, 50% NPK+50% Vermicompost, 25% NPK+75% Vermicompost) were laid out with three replications in randomized block design. The results revealed that application of 50% inorganic fertilizers + 50% FYM produced significantly higher herb and oil yield (213.3 q ha^{-1} and 157.8 kg ha^{-1} respectively), over all other treatments.

Effect of different combinations of inorganic fertilizers with FYM and Vermicompost on yield of Indian basil.

Treatments	Herb yield (q ha^{-1})	Oil yield (kg ha^{-1})
Control	121.21	90.90
Full NPK(100: 40: 40kg ha^{-1})	167.23	122.07
Full FYM 20 t ha^{-1},	193.04	140.92
Full Vermicompost 10 t ha^{-1},	159.37	114.75
75% NPK+25% FYM	175.0	127.81
50% NPK+50% FYM	213.34	157.80
25% NPK+75% FYM	186.31	137.87
75%NPK+25% Vermicompost	195.29	142.56
50% NPK+50% Vermicompost	202.0	147.47
25% NPK+75% Vermicompost	159.37	132.73
C.D at 5%	12.30	9.84

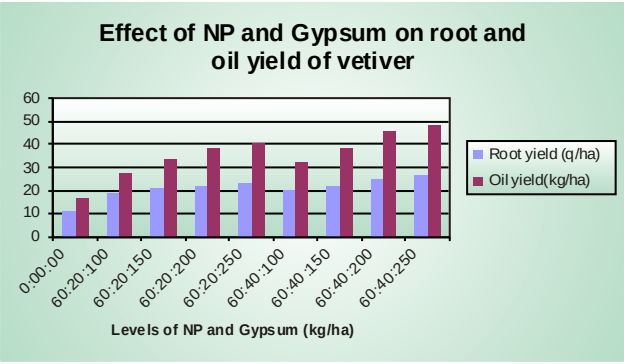
1.5. Performance of vetiver under different levels of shade (H.S.Chauhan)

Field experiment was conducted to evaluate the performance of vetiver (cv CIM-Vriddhi) under different degrees of shade (0, 25, 50 and 75%). The data showed that there was significant increase in root biomass and oil yield with decrease in shade level. Shading caused significant reduction in root biomass and oil yield. The results indicate that vetiver can be grown successfully under 40-50% shade in orchard giving approx. 20 kg oil/ha in one year.



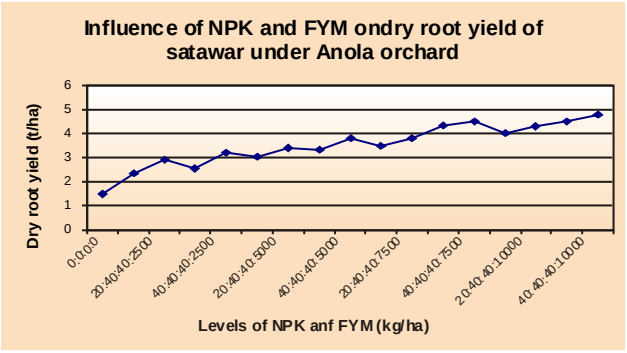
1.6. Influence of N, P and gypsum on growth and yield of vetiver under irrigated conditions (H.S. Chauhan)

Graded levels of N, P and gypsum were applied to study the growth of vetiver (cv CIM –Vriddhi). Results showed that application of N, P and gypsum significantly influenced the number of tillers/plant and root biomass yield. The significantly highest root biomass (25.4 q/ha) and essential oil yield (45.75 kg/ha) was obtained with the application of 60:40:200 kg/ha N, P and gypsum in one year crop.



1.7. Performance of satawar under anola orchard in integrated nutrient management system (H.S.Chauhan)

Results of the experiment to optimize the nutrient requirement of satawar revealed that application of 40 kg N, 30 kg P_2O_5 and 20 kg K_2O/ha along with 7.5 t/ha of FYM produced 4.35 t/ha of satawar dry root under aonla orchard (30-40% shade). Thus grower could get extra income by growing satawar in anola orchard.



1.8. Integrated nutrient management in Kalmegh (*Andrographis paniculata*) (Rajesh Verma, D.D.Patra)

The application of vermicompost and biofertilizers was able to sustain kalmegh yield at par with those obtained using only chemical fertilizers (NPK). Whereas in control treatments, where no manure or chemical fertilizers were added, there was 31% reduction in biomass yield, the maximum increase was 49%, 41%, 78% and 81% in biomass yield, numbers of branches, L: S ratio and % dry matter production, respectively, in T_2 treatments over control.

Biomass yield, number of branches, L:S (Leaf:Stem) ratio and total dry matter production as influenced by different treatments

Treatments	Total biomass yield (g)/plant	No of bran-ches/ plant	L:S (ratio)	Total dry matter production (%)/ plant
T_0 control	243.33^{d}	17.00^{d}	0.80^{d}	23.83^{c}
T_1 vermicompost	316.00^{bc}	20.66^{b}	1.26^{ab}	30.33^{b}
T_2 (T_1+ Azotobacter)	364.33^{a}	24.00^{a}	1.43^{a}	42.33^{a}
T_3 (T_1+PSB)	356.66^{a}	19.33^{bc}	1.26^{ab}	36.16^{b}
T_4 (T_1+PGPR)	316.00^{c}	18.33^{cd}	1.00^{cd}	31.66^{b}
T_5 (T_1+AMF)	320.00^{bc}	17.00^{d}	0.92^{cd}	31.83^{b}
T_6 (T_1+ T_2+ T_3+ T_4+ T_5)	343.33^{ab}	17.00^{d}	1.06^{bc}	34.00^{b}
T_7 only fertilizers (NPK)	319.66^{bc}	18.33^{cd}	0.96^{cd}	31.83^{b}

Means followed by the same letter within one parameter do not differ significantly at $p < 0.05$ by Duncan's Multiple – Range Test

PSB= Phosphate Solubilizing bacteria, PGPR= Plant Growth Promoting Rhizobacteria, AMF = Arbuscular Mycorrhizal Fungi

Project Title: Economic enabling of salt affected belt and agricultural lands in Sultanpur and Raebareli districts of U.P. by establishing "Aroma Biovillages" through technology intervention

1.9. Aroma Biovillage reaches a new height (D.D. Patra, A.K.Singh, Mansoor Alam, Alok Kalra, H.S. Chauhan)

In the third year of the project, the number of beneficiary farmers increased to more than 100, covering an area

of 50 ha on mint, basil, palmarosa, lemongrass and vetiver. New cropping systems have been developed viz. Basil + Mango, Basil + Guava, Vetiver + Mint etc. The main objective has been to receive maximum per capita productivity of land. All the farmers under the program are distilling their oils and vermicomposting the waste which is being used as a supplement to inorganic fertilizers, thereby saving about 60% of their fertilizer requirement following an integrated approach.

Vetiver + mint intercropping

Mint in farmer's field

1.10. Optimization of plant population and nitrogen fertilizer for clarysage (Mohd. Yaseen, Kambod Singh, Muni Ram)

A field experiment was conducted to study the effect of five plant densities (1.11,0.74,0.55,0.50 and 0.37 lakh plant/ha) and four nitrogen levels (0,40,80 and 120 kg N/ha) on the yield and quality of clarysage. The results revealed that significantly higher essential oil yield (17.6 kg/ha) from clarysage could be obtained by maintaining the plant density at 0.37 lakhs plants /ha at a spacing of 60 x 45 cm and fertilized with 80 kg N/ha. The oil quality was also better at above treatment having higher major essential oil constituents (linalyl acetate- 50.27% and linalool-21.061%).

Effect of nitrogen levels and plant density on fresh flower spike and essential oil yield of clarysage

Treatment	Fresh flower spike yield (q/ha)	Essential oil yield (kg/ha)
Plant density (lakh/ha)		
1.11	106.4	13.2
0.74	114.3	13.7
0.55	103.0	12.2
0.50	127.1	15.5
0.37	145.8	17.6
CD (5%)	12.9	1.5
Nitrogen levels (N kg/ha)		
Control (0)	88.4	11.5
40	113.5	14.6
80	134.5	16.2
120	140.8	15.4
CD (5%)	11.5	1.4

Field view of clarysage

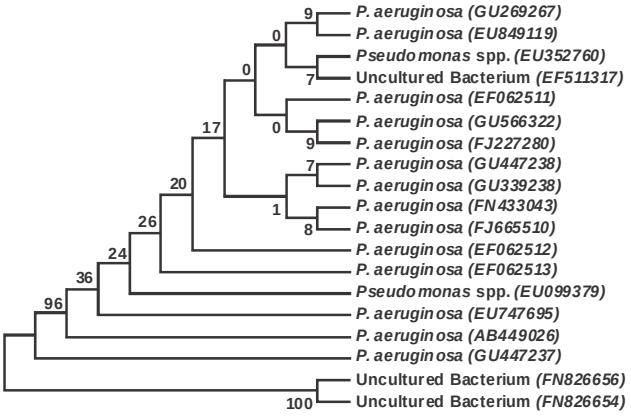
1.11. Harvest management in geranium (Mohd. Yaseen, U.B. Singh, Muni Ram)

A trial was conducted with nine harvesting stages (104, 111, 118, 125, 132, 139, 146, 153 and 160 days after planting) to assess the yield and quality of geranium. The results revealed that the herb and oil yields; L/S ratio and oil content increased with advancement of age up to 139 days, thereafter, the yield attributing characters decreased. Best quality of oil (Citronellol 29% and Geraniol 22%) was also obtained at this stage.

1.12. Isolation, characterization and antimicrobial activity of metabolite from *Pseudomonas aeruginosa* SD12, a new strain from tannery waste polluted soil (D.D. Patra, Mohd. Anwar, Sukhmal Chand, Mansoor Alam, Abdul Samad, S.K. Srivastava)

Pseudomonas aeruginosa SD12, a new strain, isolated from the tannery waste polluted soil was identified on the basis of morphological, biochemical and physiological characteristic features and 16S rRNA sequence analysis. The strain has shown 99% homology

with *P. aeruginosa*. It has shown antifungal activity against wide range of plant pathogens. The strain produced considerable amount of phosphatases, cellulases, proteases, pectinases and indole-3-acetic acid (IAA). The strain also produced substantial amount of hydroximate siderophore which was highly effective in making complexes with the trace elements like Fe^{2+}, Cu^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+}. It helps in bioremediation of heavy metals like Ni^{2+} Pb^{2+}, Cd^{2+}, Cu^{2+} and Sn^{2+}. A bioactive metabolite was isolated from *P. aeruginosa* SD12 and characterized as 1-hydroxyphenazine (1-OH-PHZ) by nuclear magnetic resonance (NMR) spectral analysis. The metabolite produced antimicrobial activity against various phytopathogens of agricultural importance as well as human pathogens. The metabolite, 1-hydroxyphenazine from *P. aeruginosa* SD12 displayed appreciable antimicrobial activity and even against methicillin resistant *Staphylococcus aureus* (MRSA), which is the first report.



Phylogenetic tree analysis of *Pseudomonas aeruginosa* SD12 based on the nucleotide sequence of 16s RNA gene. The branching pattern w as generated by the neighbour joining (NJ) method (MEGA 4 version) and accession numbers are indicated in parentheses.

1.13. Investigation on the efficacy of Zn application to mitigate the influence of cadmium on lemongrass (Amitabh Chattopadhyay)

The results of the experiment to assess the influence of cadmium on lemongrass and to examine the efficacy of $ZnSO_4$ to mitigate the influence of applied Cd on the herb and oil yield of the plant has revealed an initial increase in the herb and oil yield of lemongrass due to sole application of 25 ppm of cadmium followed by a steady reduction of 20% in those parameters with increased application of Cd up to 200 ppm. Application of $ZnSO_4$ at 30 kg/ha was found effective to produce more oil yield in all the treatments including control except in the initial treatments of Cd application (25 ppm) where it was not effective. The maximum recovery of 57 % in the oil yield of Lemongrass was recorded in the soil spiked with 100 ppm of Cd as compared to soil treated with Cadmium alone at the same level of addition.

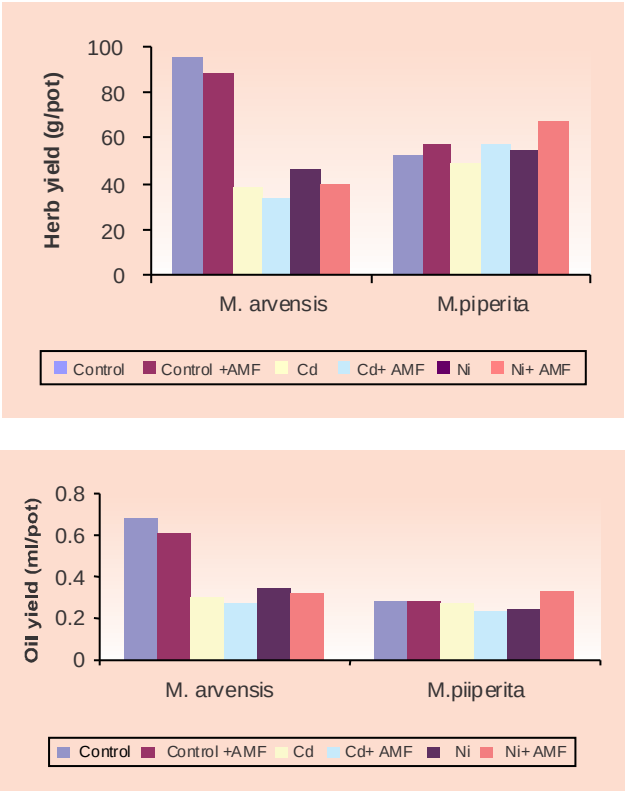
1.14. Organic matter as an amendment to mitigate the influence of varying levels of chromium in soil on Palmarosa (Amitabh Chattopadhyay)

The results of the first harvest data of the ongoing experiment on Palmarosa to study its tolerance level towards graded level of chromium application in soil and to assess the potential of organic matter to mitigate the influence of chromium on the herb and oil yield of the plant has indicated that Palmarosa has capacity to tolerate available Cr in soil up to 19 mg/kg above which reduction in plant biomass took place. The maximum reduction of which was 67% in soil having 28 ppm of Cr as compared to control with no external Cr application.

Soil spiked with higher levels of chromium and geranium spent compost recovered the herb yield of palmarosa by 46% as compared to the soil spiked with same level of Cr alone.

1.15. Studies on the effects of heavy metals (Cd and Ni) and arbuscular mycorrhizal (AM) fungi on growth and yield of mint species (Arun Prasad)

Interactive effects of heavy metals (100 mg of Cadmium and Nickel and arbuscular mycorrhizal (AM) fungi (*Glomus intraradices*) on the yield, chemical composition of essential oil and metal accumulation in *Mentha arvensis* and *Mentha piperita* was investigated in a pot experiment. The study suggests that *M. piperita*- AM symbiosis could be used as plant- based strategy for revegetation and phytostabilization of highly Cd and Ni contaminated soils.



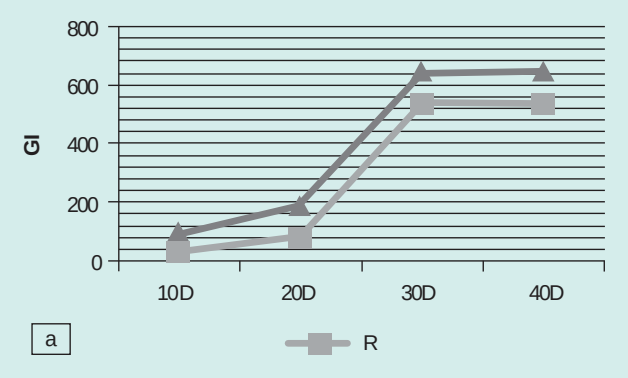
Effect of heavy metals (Cd and Ni) and AM fungi on the herb and oil yield of *Mentha arvensis* and *M. piperita*

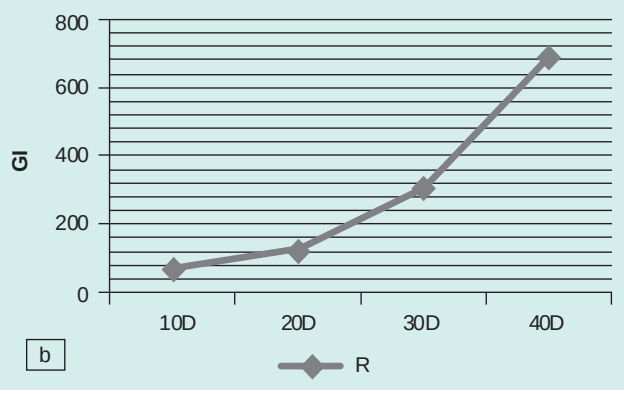
2. BIOTECHNOLOGY

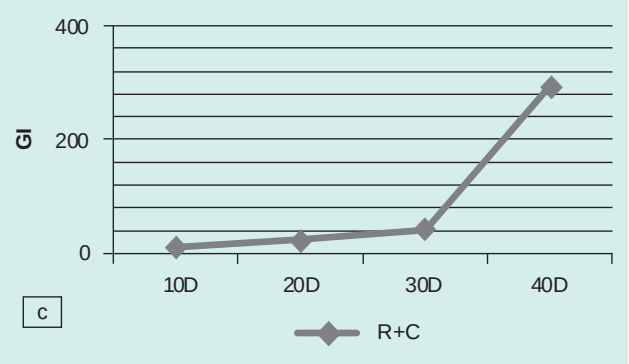
PLANT BIOTECHNOLOGY

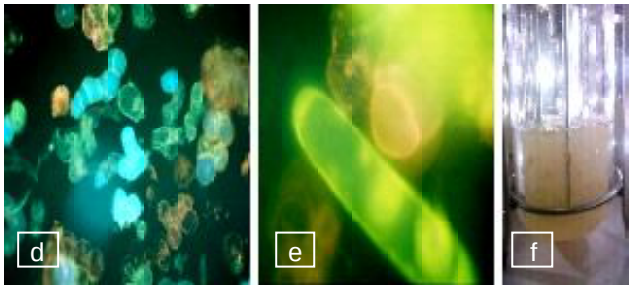
2.1. Mixed cell suspension cultures of *Catharanthus roseus* and *Rauvolfia serpentina* scaled-up from shake flask to bioreactor for pathway complementation studies towards *in vitro* metabolite production (A.K. Mathur, M.M. Gupta, R.K.Verma)

The novel approach towards pathway complementation through mixed cell suspension culture technology was up-scaled from shake flask to 7 litre air-lift bioreactor level. The growth kinetics of two partner cell types in their respective growth medium in shake flask culture indicated that the cultures entered the exponential phase with a G.I. of 540.67 and 637.90 on 30th day with no substanial gains during next 10 days. The *R. serpentina* cells in *C. roseus* medium took a longer time to acclimatize in the new medium with a GI of only 308.12 on 30th day but acquired comparable growth on 40th day in the two different media. In mixed culture mode in 1:1 ratio *C. roseus* + *R. serpentina* cultures attained a final GI value of only 292.29 on 40th day with cell ratio of 3:2 between *Catharanthus* and *Rauvolfia* cells in this first culture cycle. The mixed culture upon sub-culturing for 4-5 cell doubling passages showed gradual improvement in proliferation rates and acquired a consistent GI values









Time-course biomass accumulation (GI) pattern in cell suspensions of (a) Rauvolfia-(R), (b) Catharanthus-(C) grow n individually and (c) in mixed culture (R+C) modes in different medium (d, e) RITC and FITC stained partner cells in mixed culture phase; (f) bioreactor up-scaling of cells in air lift fermentor.

of around 550. The crude alkaloid contents of individual and mixed cell suspension cultures were found to be 0.31, 0.34 and 0.42 % dry weight in case of *Catharanthus*, *Rauvolfia* and *Rauvolfia* + *Catharanthus* cultures, respectively. A 20-25 fold increment in biomass of mixed cultures with a CPV= 47ml/ 100ml suspension was achieved through a 30 days cycle in 7 litre bioreactor.

2.2. Development of a two tier flask system for CO_2 enrichment of culture environment to screen photo-mixotrophic to photo-autotrophic cell lines of *Catharanthus roseus* (A.K. Mathur)

Selection of photoautotrophic cell lines in *Catharanthus roseus* has been achieved under CO_2 enrichment and high light intensity using a two tier culture flask system. Initial screening attempts under gradual sucrose lowering regimes from 3.0% to 1.0% in the medium indicated a narrow base for desired selection at both shoot and callus levels of growth. Mutagenesis of the wild line callus tissue with ^{60}Co γ-radiation at 2.0-20.0 kR dose, followed by continuous screening under sucrose starvation has yielded eight putative variant lines capable of growing on medium with 0.5% sucrose. The best growth and survival in case of irradiated callus line

was obtained from a sector recovered from 15 kR radiation dose. This line, designated as 15kR/ 3-3-1/ 0.25, beside fast proliferation is also characterized by the development of a dark green morphology. Further efforts to improve biomass accumulation in this selected line are under way.

2.3. Complete transgenic protocol for whole plant-level metabolic engineering in *Catharanthus roseus* developed at CSIR-CIMAP (A.K. Mathur)

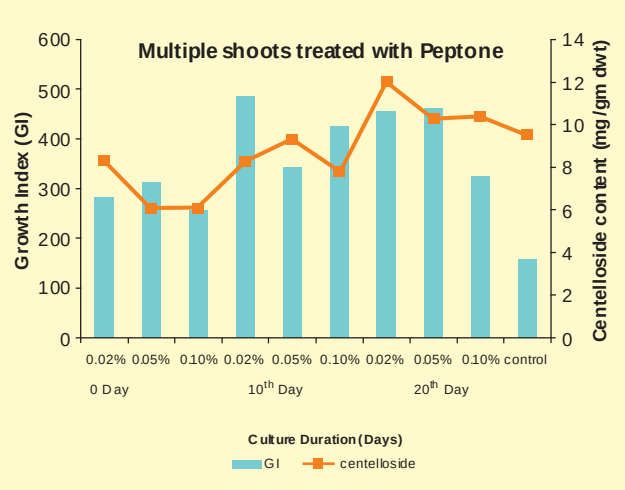
An efficient transgenic protocol for pathway engineering at whole plant level in *Catharanthus roseus* has been developed for the first time. *Agrobacterium tumefaciens*-mediated transformations were carried out using LBA4404 strain harboring a binary vector pBI121 with p35SGUS-INT gene construct. Highest transformation efficiency of 1.4 transgenic shoots/responded explant was obtained when pre-plasmolysed leaves, pre-incubated on shoot bud induction medium for 10 days, were subjected to a SAAT treatment for 30 s prior to transformation (Table below). Using a selection medium containing 50 mg/L kanamycin, the transformants grew into micro-shoots and formed roots on a hormone-free half strength MS medium. The transgenic nature of the regenerated plants was confirmed by PCR amplification of uidA gene and GUS histochemical assay.

2.4. *In vitro* enhancement of biomass and centelloside production in multiple shoot cultures of *Centella asiatica* by biotic elicitation with peptone (Archana Mathur, M.M. Gupta)

Peptone at various concentrations was found to elicit biomass as well as total centelloside production in *in vitro* multiple shoot cultures of *Centella asiatica.* The high growth Index (GI 446) and maximum centelloside (12 mg/gm dwt) was obtained upon feeding the 20 days old cultures with 0.02% peptone.

2.5. Optimisation of centelloside production in *Centella asiatica* under glass house conditions (Archana Mathur, N.K.Srivastava A.K.Mathur, M.M.Gupta, G.C.Uniyal)

Hydroponics culture-based cultivation of *Centella asiatica* optimized for the first time to address the problem of heavy metal toxicity and microbial load in this herb collected from the wild or field based cultivation approach. Growth kinetics of hydroponically grown plants maintained in the glass house was monitored over a period of 70 days.



Frequency of shoot bud and plant regeneration from leaf explants of *Catharanthus roseus* transformed with LBA 4404 strain of *Agrobacterium tumefaciens* harboring the plasmid *p35SGUS-INT*

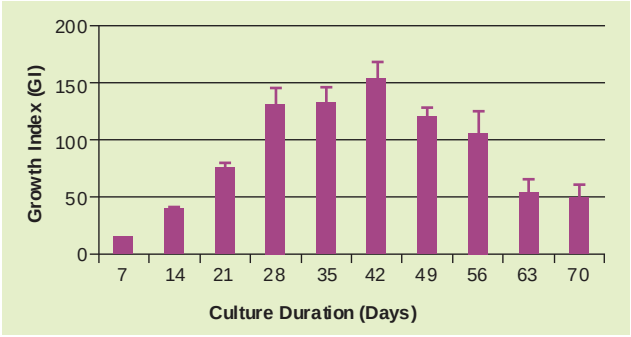
Exp. Set up	% explants showing s. b. regeneration	No. of s. b./ responded explant	No. of s. b. shifted to selection medium	No. of micro-shoot obtained on selection medium	% shoots showing *GUS* positive staining	Transformation efficiency*
1	29.1	3.2	12	5	2	0.5
2	49.4	5.7	23	11	3	0.7
3	21.3	1.8	NR	14	11	1.4
4 (Control)	71.3	6.3	20	0	0	0

1 Pre-plasmolysed leaf explants after SAAT treatment were co-cultivated on MS basal for 5 days, shifted to SBM for shoot bud regeneration and developed shoot buds were transferred to the selection medium (SBM+50 mg kanamycin l^{-1})

2 Pre-plasmolysed leaf explants were pre-incubated on SBM for 10 days prior to SAAT treatment, co-cultivated on SBM for 5 days followed by shifting to fresh SBM for shoot bud regeneration and developed shoot buds were then transferred to the selection medium

3 Pre-plasmolysed leaf explants were pre-incubated on SBM for 10 days prior to SAAT treatment, co-cultivated on the selection medium for shoot bud regeneration and micro-shoot development

4 Pre-plasmolysed non-transformed explants were plated on SBM for shoot bud and micro-shoot formation
(s.b., shoot buds; * No. of transgenic shoots recovered/ responded explant)



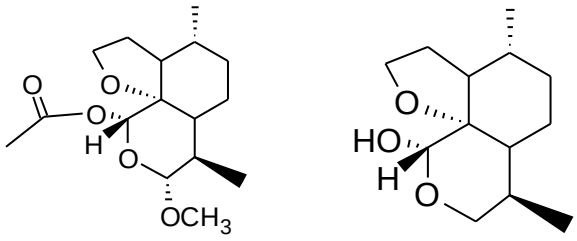
Grow th and centelloside production in hydroponically established plants of *C. asiatica*. A. Hydroponic culture, B. Grow th curve of hydroponically established plantls of *C. asiatica* at the interval of 7 days

Quantitative analysis of the centellosides produced revealed the presence of major centelloside i.e., madecassoside, asiaticoside, madecassic acid and asiatic acid in these plants in appreciable amounts. These observations clearly provide evidence that hydroponic systems can be effectively used for the efficient production of *C. asiatica* for quality herb.

Project Title: Plant cell suspension culture based biotransformation

2.6. Up-scaling and characterization of biotransformed product of α and β-artemether using cell suspension cultures of *Glycyrrhiza glabra* and *Lavendula officinalis* (Archana Mathur, R.S.Bhakuni, A.K.Mathur)

The procedure for bioconversion of both α and β-artemether by cell suspension cultures of *Glycyrrhiza glabra* and *Lavendula officinalis* was upscaled using 250 mg substrate. Two derivatives were obtained in given condition from both forms of artemether i.e. α and β-artemether and were characterized as THF-acetate derivative of α and β-artemether and 5-hydroxy ring contracted deoxoderivative of α and β-artemether.



(a) (b)

THF- acetate derivative of α-artemether (a) and 5-hydroxy ring contracted deoxoderivative of artemether (b) obtained following biotransformation of artemether by *G. glabra* and *L. officinalis* cell suspension cultures.

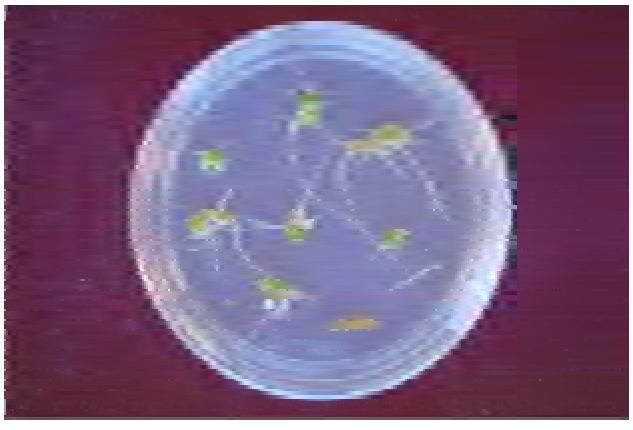
2.7. Direct regeneration studies in Tagetes and Pyrethrum (Laiq-ur-Rahman)

A protocol has been developed for differentiation of shoot buds directly from different explants of *Tagetes erecta* like cotyledonary leaf, rachis, hypocotyls etc. To increase the multiplication rate of shoot buds various combinations of 6-benzyl amino purine (BAP) and Gibberellic acid (GA3) were tried. The plants were transferred in pots and showed 60% establishment. Similarly in pyrethrum, direct regeneration was tried from leaf and petiole explants. MS media with different concentrations and combinations of BAP and TDZ were used.

2.8. Genetic transformation studies in Pyrethrum and *Tagetes* through *Agrobacterium rhizogenes* (Laiq-ur-Rahman, Suchitra Banerjee)

Genetic transformation studies in Pyrethrum and Tagetes through *Agrobacterium rhizogenes* were

Hairy root induction in Pyrethrum



Hairy root induction in *Tagetes*

performed for generating transgenics and producing secondary metabolites that are normally biosynthesized in the roots of differentiated plants. Induction of hairy roots in pyrethrum was done using *Agrobacterium rhizogenes*. Maximum frequency of 72% of root induction was observed in leaf explants, while only 27% petiole explants showed hairy root initiation. In case of *Tagetes* roots were induced from leaf explants of *in vitro* grown plants. More than 80% explants showed root initiation after 15 days of infection. These roots were grown in liquid MS media and showed profuse growth.

2.9. Functional genomics of volatile terpenoid metabolism in curry leaf, *Murraya koenigii* (Dinesh Nagegowda)

This study is aimed at isolation of novel terpene synthase genes in *M. koenigii* with a long term goal of understanding the regulation of mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways and their role in essential oil biosynthesis. Four full length terpene synthases have been isolated by degenerate PCR and RACE strategy. Phylogenetic analysis was carried out by using the deduced amino acid sequences for all the genes and the percent homology between each protein was calculated. The putative subcellular localization of each protein was predicted *in silico*. All four genes have been subcloned into pET vectors and were expressed in *Escherichia coli* to obtain the recombinant protein. The detailed functional characterization of the isolated terpene synthases for their ability to convert geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP) into monoterpenes and sesquiterpenes is to be completed.

Project Title: Genotype designing for specialty/ opportunity crops in MAPs

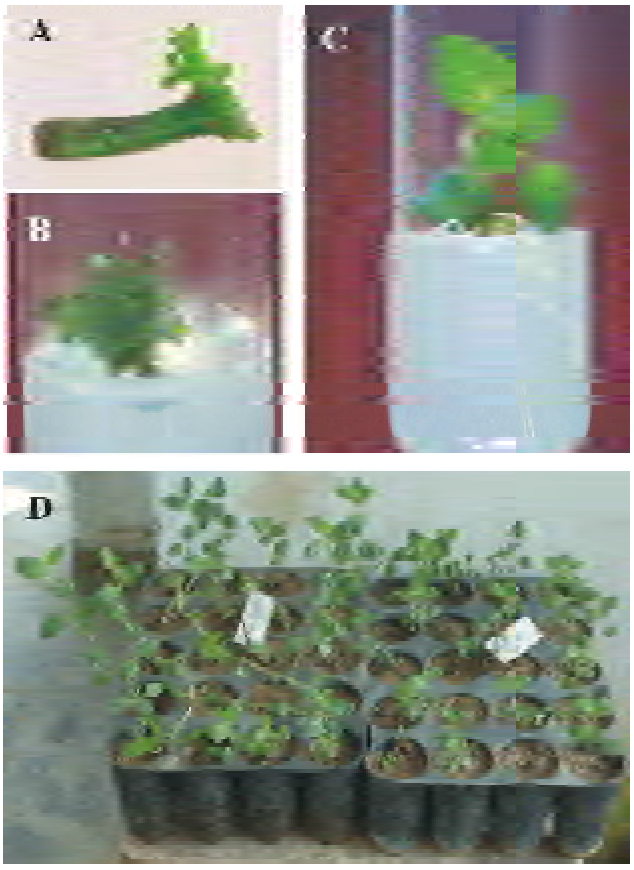
2.10. Efficient multiple shoot regeneration in *Mentha piperita* (Sanjog Thul)

An efficient protocol for direct multiple shoot regeneration from internodal explants has been defined in peppermint (*Mentha* x *piperita* var. Indus). *In vitro* regenerated shoots of peppermint were cultured in MS medium supplemented with different cytokinins. In hormonal assay 3.0 mgl^{-1} zeatin or 6-isopentenyl adenine independently supplemented to half strength MS medium exhibited multiple shoot regeneration. A maximum of 85% *in vitro* cultured explants showed regeneration and multiple shoot formation response on MS medium supplemented with zeatin. Internodes with regenerated multiple shoots were transferred to half - strength MS medium without supplementing any plant growth hormone for shoot elongation and rhizogenesis. The rooted plants successfully acclimatized and grew to maturity under glasshouse conditions.

Effect of cytokinins on morphogenetic response in *M. piperita*.

Cytokinin (mgl^{-1})	Morphogenetic response (%)		
	Z	TDZ	2-iP
0.0	-	-	-
0.1	-	-	R (25)
0.5	-	R (35)	R (50)
1.0	MS (25)	-	R (40)
2.0	MS (35)	-	MS (35)
3.0	MS (85)	-	MS (50)

MS-multiple shooting; R - rooting; - no response



In vitro shoot multiplication and plantlet development in *M. piperita* var. Indus. (A) Direct multiple shoot bud development from cut ends of internodal explants; (B) multiple shoots after 4 weeks of culture; (C) Root induction in elongated adventitious shoot; (D) *in vitro* raised hardened plants.

Project Title: Basic research towards path breaking MAPs sciences

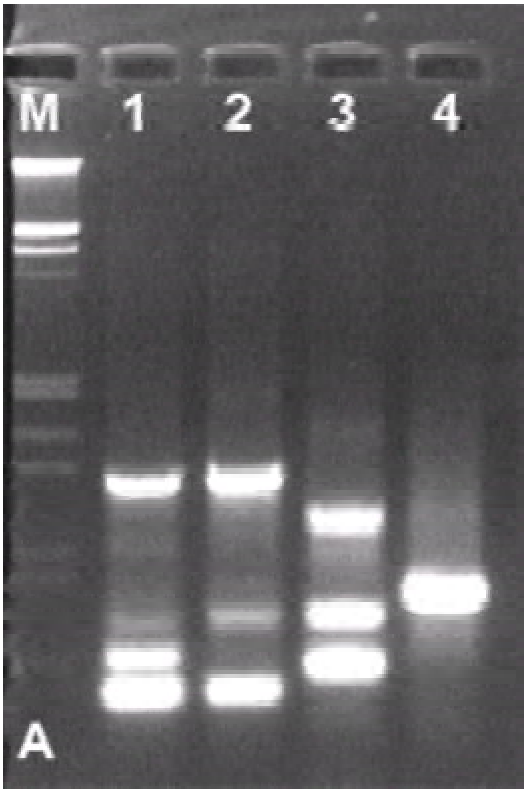
2.11. Genetic diversity analysis of *Sida* species using ISSR markers (Sanjog Thul)

The present method is a modification of CTAB method that can be used for diverse plants. Isolated DNA samples were successfully amplified through PCR amplification using ISSR markers. For genetic fingerprint investigation, 10 ISSR primers generating reproducible banding patterns were used. Of the total 63 amplicons generated, 62 were recorded as polymorphic. Genetic

similarity index deduced from ISSR profiles ranged from 12 to 51%. Based on similarity index, *Sida acuta* (SA) and *S. rhombifolia* (SR) found to be most similar (51%).

Average genetic similarity index based on ISSR analysis of Sida Species.

Species	SA	SR	SF	ST
SA	1			
SR	0.51	1		
SF	0.26	0.27	1	
ST	0.12	0.19	0.24	1



ISSR banding profile of *Sida* species generated by primers 826 and 844. Lane M: Lambda DNA double digested (*EcoR* I+*Hind* III) size makers, (**A**) PCR products of various Sida sp. with primer UBC 826.

2.12. Expression of RAPD primers towards identification of species-specific molecular markers in *Sida* (Sanjog Thul)

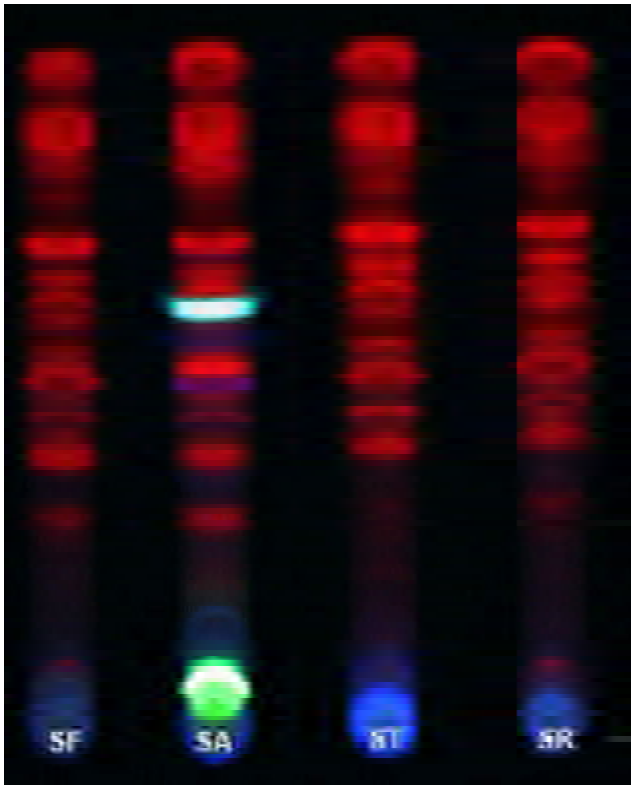
Authentication of medicinal plants at genetic level is a critical step in the use for both research purposes and commercial preparation. As is known, morpho-chemical characters are influenced by environmental factors; while DNA markers are least affected by environment but gets linked to genotypes for their genomic constitution. Four species viz., *Sida acuta*, *S. rhombifolia*, *S. cordifolia* and *S. cordata* of *Sida* complex were used. A set of 20 primers were screened to identify species-specific molecular markers. Eight primers so far expressed with reproducible bands.

BIOTECHNOLOGY AND CHEMICAL SCIENCES

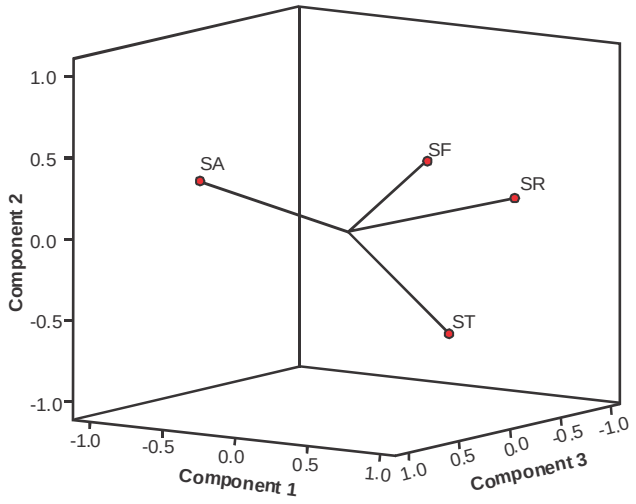
Project Title: Basic research towards path breaking MAPs sciences

2.13. Genetic and chemical diversity analysis of *Sida* complex by ISSR markers and chemical fingerprinting (Sanjog Thul, S.C. Singh and Karuna Shanker)

Isolated DNA samples were PCR amplified using ISSR markers and degree of genetic diversity among the different species of *Sida* is compared with that of chemical diversity. For genetic fingerprint investigation, 10 ISSR primers generating reproducible banding patterns were used. High number of species-specific bands played pivotal role to delineate species at genetic level. Investigation based on HPTLC fingerprint analysis revealed 23 bands representing characteristic chemicals, and similarity index ranged from 73 to 91%. Prominent distinguishable bands were observed only in *S. acuta*, while *S. cordifolia* and *S. rhombifolia* shared most bands making them difficult to identify on chemical fingerprint basis. This report summarizes the genotypic and chemotypic diversity and the use of profiles for authentication of species of *Sida* complex.



Chemical fingerprint profiles of *Sida* species on HPTLC plates detected by UV illumination.
(SF) *S. cordifolia*, (SA) *S. acuta*,
(ST) *S. cordata*, (SR) *S. rhombifolia*.



Chemical fingerprint based PCA 3D plot depicting the relationship among the Sida species.

2.14. Production of Bio-chemicalsand Bio-fuel from Spent Aromatic Biomass (Ram Rajasekharan, Prasant K Rout, Ashween D. Nannaware)

In order to meet the energy demand of the world, lignocellulosic biomass (aromatic waste) has come up as a prime candidate for the production of conventional hydrocarbon liquid transportation fuel and petrochemicals products.

Our research specifically focuses on the development of process intensification technology for glucose production from spent aromatic waste biomass using physicochemical process, in which the spent aromatic biomass obtained after distillation is utilized for glucose production by physicochemical process.

Our research also focuses on the conversion of the aromatic waste biomass to value added chemicals like hydroxy methyl furfural (HMF), levulinic acid (LA) and furfural through solid metal chloride catalyst / acid combination hydrolysis. Main advantages of this processes is that it requires less reaction time and temperature. The chemicals produced from this reaction can be used as an alternative high calorific value fuel. Besides fuel, these can also serve as platform for the production of various organic chemicals with applications in the polymer, fuel additive, pesticide and other chemical industries.

Our future plans are underway to scale up acid catalyzed reaction / acid combination hydrolysis for the production of levulinic acid and HMF in a mobile processing unit in CSIR-CIMAP.

MOLECULAR BIOPROSPECTION

2.15. Identification of drug targets in *Propionibacterium acnes* (Dharmendra Saikia)

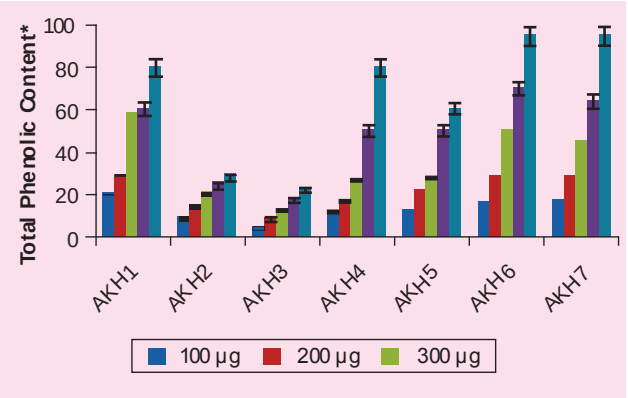
Propionibacterium acnes belongs to the human cutaneous, non-spore-forming, gram positive anaerobic, pleomorphic rod whose end products of fermentation include propionic acid. Antibiotics are becoming less and less useful as resistant *P. acnes* are becoming more common. Genomic data has revealed many genes that encode enzymes in metabolic pathways and virulence factors including sialidase that are involved in degrading host tissue and inducing inflammation. Lactate dehydrogenase enzyme involved in anaerobic respiration, catalyses the inter-conversion of pyruvate and lactate in *P. acnes*. For developing target based assay we have chosen sialidase and lactate dehydrogenase as drug targets as both the enzymes play a very significant role in the pathogenesis of *P. acnes*.

2.16. Development and standardization of bioassay for ornithine decarboxylase (Suaib Luqman, A.S. Negi, Abha Meena)

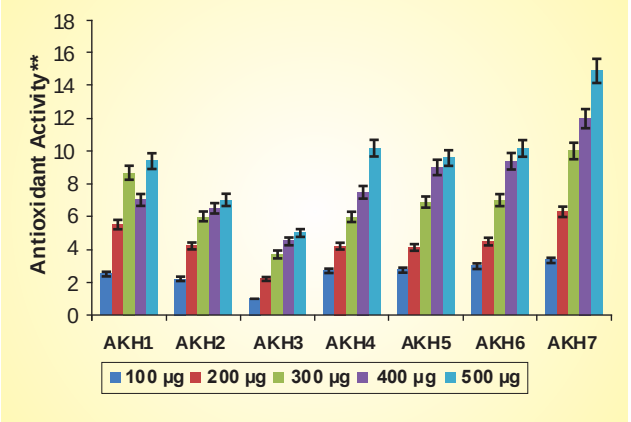
An assay has been developed and standardized for evaluation of natural products and their derivatives inhibiting ornithine decarboxylase activity, a validated target in infectious diseases and cancer.

2.17. Exploring antiproliferative and antioxidant activities of *Juglans regia* fruit (Suaib Luqman, A.S. Negi)

Cancer chemopreventive action of various fractions of walnut (*Juglans regia* L., Family: Juglandaceae) extract has been explored. Chloroform and ethyl acetate fractions exhibited high level of antiproliferation against



Total Phenolic Content estimated in terms of gallic acid equivalence.



Antioxidant activity estimated in terms of ascorbic acid equivalence.

liver cancer cell line. Exhibiting high phenolic content, antioxidant activity, and potent antiproliferative activity, walnut may act as cancer chemopreventive agent.

2.18. Neoflavonoids as cancer chemopreventive agent (Suaib Luqman, Abha Meena, A.S. Negi)

Several neoflavonoids pharmacophore were studied *in silico* using computer aided molecular docking studies. All the analogues were synthesized from piperonal, a precursor phytomolecule and docked with targets related to cancer. Based on the docking score, NFkB1, NFkB2, aromatase, quinone reductase 1, quinone reductase 2, ornithine decarboxylase, iNOS and protein kinase C are suggested as valid targets for these compounds; however additional investigation of chemopreventive or chemotherapeutic potential is required for neoflavonoids.

Project Title: Exploitation of India's rich microbial diversity

2.19. Characterization of bioactive endophytic bacteria (M.P. Darokar, Alok Kalra)

Antibacterial activity of culture supernatants and extracts of 14 endophytic bacteria isolated from the leaves of *Pelargonium graveolans* were tested against 9 different human pathogenic bacteria through *in-vitro* assay. The culture supernatant of three endophytes coded as Ger-3, Ger-9 and Ger-14 possessed antibacterial activity against one or the other test microorganisms. The methanolic extract obtained through diaion HP-20 from these endophytes also exhibited activity against the test microorganisms. Extract of Ger-3 exhibited activity against *Micrococcus luteus, Streptococcus mutans* and *Staphylococcus aureus* including methicillin resistant strains. This extract also exhibited anticancer activity against human oral cancer cell line KB with IC 50 and IC 90 values 50 and 105 µg/ml respectively. Considering the activity profile exhibited by the endophyte Ger-3, it was further subjected to taxonomic identification through 16S rDNA sequence analysis. This endophyte showed maximum similarity (99%) with *Bacillus* spp. Accession number-HQ112283 (NCBI-Gene Bank).

Project Title: *In-vitro* and *in-vivo* biological activities including drug targets and pharmacological studies

2.20. Antimycobacterial phytomolecule khusimol from *Vetiveria zizanioides* (M. P. Darokar, Anirban Pal, Dharmendra Saikia, Ashok Sharma, S.K. Srivastava)

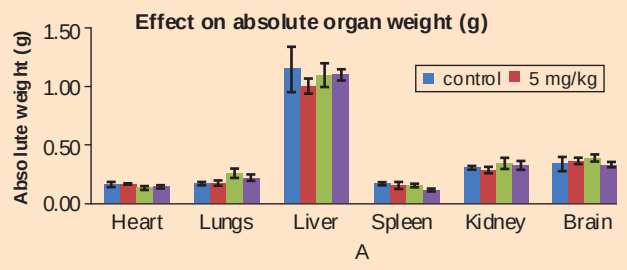
A phytomolecule khusimol that was identified and purified as antibacterial agent from the plant *Vetiveria zizanioides*, was further studied and found to be active against virulent strain H37Rv of *Mycobacterium tuberculosis*. Khusimol, in *in-vitro* assay was observed to inhibit the super coiling induction activity of bacterial DNA gyrase. Binding affinity values in docking experiments of two receptors 1ABP4 (gyrA) and 1EI1 (gyrB) with khusimol and known DNA gyarase inhibitors were comparable. This molecule exhibited no mortality and morbidity even at higher dose of 2000 mg/kg body weight during acute oral toxicity study.

2.21. Acute oral toxicity study of TeT-31 in Swiss albino mice (Debabrata Chanda, A.S. Negi)

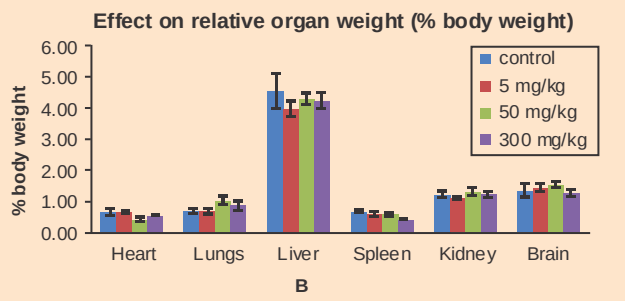
In view of potent anti-TB activity of TeT-31 in *in-vitro* model, acute oral toxicity of the same was carried out in Swiss albino mice for its further development into drug product. Experiment was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline No 423 (1987).

The experiment showed that TeT-31 is well tolerated by the Swiss albino mice up to the dose level of 300 mg/kg body weight as a single acute oral dose. However, sub-acute and or chronic experiment with the test drug needs to be carried out to look for any adverse effect on repeated exposure to the test drug.

Effect of TeT-31 as a single acute oral dose at 5, 50 and 300 mg/kg body weight on absolute (A) and relative organ weight (B) in Swiss albino mice.(n=4, Non significant changes were found compared to control).



A



B

2.22. Developing non-invasive method for rapid and simple determination of drug concentration in animal plasma for pharmacokinetic analysis (P.V. Ajayakumar, Debabrata Chanda, Anirbal Pal)

In this experiment, we have studied the pharmacokinetics of nimesulide in rabbits using *in vivo* measurements of nimesulide content in the plasma samples, collected after an i.p injection, through FT – NIR spectroscopy analysis combined with partial least squares modeling (PLS). The results are validated through HPLC analysis. The pharmacokinetic parameters calculated using FT-NIR spectroscopy and HPLC are significantly similar and error percent is below

than the acceptable limit of 6%. This is the first report of demonstrating the capability of FT-NIR measurements in the pharmacokinetic and bioavailability studies. The FT-NIR based PLS model is much faster than the available routinely used methods, highly convenient as no extraction procedure is required, economical, no solvent is required for the analysis and eco-friendlier than the other methods used in the evaluation of drug concentration in biological matrix.

Project Title: Antimalarial screening programme

2.23. Curcumins inhibit Sorbitol-induced hemolysis of *Plasmodium falciparum*-infected Erythrocytes (M.P. Darokar, B.S. Sisodia, A.S. Negi)

Curcumin and its natural analogues demethoxycurcumin and bisdemethoxycurcumin isolated from *Curcuma longa* were investigated for their effect on new permeation pathways of *Plasmodium falciparum*-infected erythrocytes by sorbitol induced hemolysis. We could demonstrate for the first time that all the three compounds inhibited the process with IC_{50}s in the range of 5.48-7.86 µM and this activity was found to be correlated with their antiplasmodial activity. Our study suggests that curcumins may exert their antimalarial effects by interfering with new permeation pathways.

Antimalarial Activity and effects on Sorbitol-induced Hemolysis of Curcumins

Test Sample or Standard drug/inhibitor	Antiplasmodial activity against NF-54 clone of *P. falciparum* (µM)	Sorbitol-induced hemolysis	
	MIC [a]	% Hemolysis at 10 µM	IC_{50} (µM)
Curcumin (Cur-A)	16.9	18.9	5.48
Demethoxycurcumin (Cur-B)	18.5	28.6	6.12
Bisdemethoxycurcumin(Cur-C)	20.2	45.9	7.86
Chloroquine	0.2	ND [b]	ND
Artemisinin	0.1	ND	ND
Furosemide	-	51.3	10.27

[a] Minimum Inhibitory Concentration required to inhibit development of ring stage parasites into schizonts, data are means from two independent experiments performed in duplicate.

[b] ND, Not determined.

2.24. Exploring functional genomic basis for medicinal properties (*Dosha-balancing*) of some plants used in Ayurveda (Ashutosh Shukla, A.K. Shasany)

Transcriptomic approaches were employed for exploring the molecular basis for classification of plants according to their *dosha-balancing* properties mentioned in Ayurveda. Twelve plants used in the study were - *Piper longum*, *Ocimum sanctum*, *Acorus calamus* and *Plumbago zeylanica* for *Kapha dosha*; *Cynodon dactylon*, *Vetiveria zizanioides*, *Asparagus racemosus* and *Santalum album* for *Pitta dosha*; *Curcuma longa*, *Sida cordifolia*, *Commiphora wightii*, and *Ricinus communis* for *Vata dosha*. Earlier, cDNA-AFLP analyses of the Ayurveda-defined active tissues of selected plants in a particular dosha group had not shown much similarity at the transcript level. Another approach, SESA (Specific Expression Subset Analysis), based on subtractive hybridization was subsequently used to validate the results of cDNA-AFLP. For each plant, the tester was the Ayurvedically active tissue/part and the driver was the mRNA from the other major tissues of the plant. A total of 803 subtractive ESTs were generated from the twelve plants that yielded 150 unigenes that were analysed for homology-based annotation using BLASTx tool with the nonredundant (nr) database. The ESTs have been submitted to dbEST division of GenBank (NCBI). SESA results indicated that there is little similarity at the transcript level among the plants belonging to the same dosha group and validated the results obtained in the cDNA-AFLP analysis, thereby disproving the hypothesis that Ayurveda-defined active tissues of taxonomically different plants belonging to the same *dosha shamak* group have similar transcripts expressed in them.

BIOTECHNOLOGY AND PLANT BREEDING

2.25. Comparative genetic analysis of trichome-less and normal pod genotypes of *Mucuna pruriens* (Kewanch) (S.S. Dhawan, M.P. Darokar, R.K. Lal, H.O. Misra)

Genetic stocks of Kewanch (contains L-DoPA, a neurotransmitter precursor) were screened for trichome less, high seed yield and L-DoPA content. The highest yielder trichome-less elite strain was selected and differentiated on the basis of PCR based DNA fingerprinting method RAPD by using deca-nucleotide primers. The analysis of amplification spectra of 408 bands obtained by 56 primers clearly demonstrated the differentiation of selected accession with trichome-less pods and established the effectiveness of this molecular tool for establishing the distinct identity of selected accession of *Mucuna pruriens*.

2.26. Characterization of transcriptional regulators/ regulatory regions from *Artemisia annua* (Vikrant Gupta)

In an attempt to study the molecular aspects of the development of glandular trichomes and production of secondary metabolites in *Artemisia annua*, homologs of *MIXTA* (a glandular trichome development-related gene of *Antirrhinum majus*) were identified in the EST

database of *Artemisia annua* in NCBI. Two putative *MIXTA* homologs were amplified by RT-PCR and cloned. One of them represented the complete ORF of MIXTA-like protein.

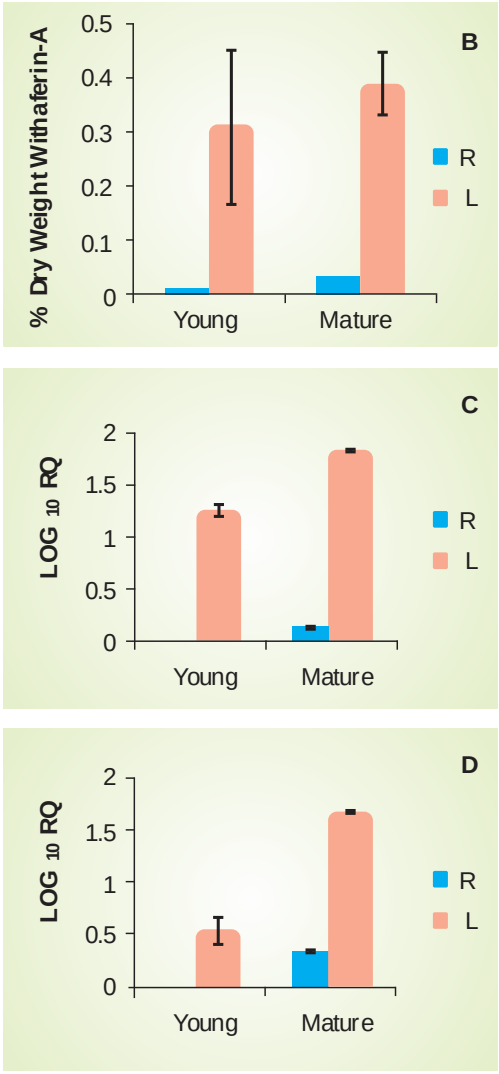
2.27. Characterization of promoter of a WRKY homolog from *Artemisia annua* (Vikrant Gupta)

A *WRKY* homolog was identified in the in-house generated EST pool of a trichome-enriched cDNA library from *Artemisia annua*. An overexpression construct was made in pBI101 which was mobilized in *Agrobacterium tumefaciens*. Heterologous plant transformation (*Arabidopsis* and tobacco) is in progress for the functional analysis of the identified *WRKY*. The genomic region upstream to the *WRKY* cDNA has also been amplified and cloned. After the complete sequencing of the cloned genomic fragment, it was found to be 519 bp long which included partial *WRKY* cDNA sequence. A putative TATA Box was found at 25-30 bp upstream of the putative transcription start site. In order to analyze the strength and tissue-specificity of *WRKY* promoter, further cloning in pBI101 (having a promoter-less *GUS* gene) is in progress.

2.28. Abundance of sterol methyl transferase (*smt1*) and obtusifoliol-14 α-demethylase (*odm*) transcripts correlated to metabolite (withaferin-A) profiling in *Withania somnifera* (Ashutosh Shukla, M.M. Gupta, A.K. Shasany)

Earlier we have reported two full-length genes sterol methyl transferase (*smt1*) and obtusifoliol-14á-demethylase (*odm*), whose products are predictively involved in the sterol biosynthetic pathway leading to withanolides. In this study, the abundance of *ODM* and *SMT1* gene transcript was found to be always higher in the leaves as compared to the roots of *W. somnifera* ('CIM-Poshita') and increased in both the organs as the plant grew from a young (≤ 6 week-old) stage to a mature (12-18 week-old) stage. The withaferin-A content in the leaves was also found to be significantly higher than that in the roots at both the stages and increased concomitantly with the transcript abundance of *ODM* and *SMT1* in both the organs as the plant matured. This is the first study on the withanolide biosynthetic pathway gene expression profiles in conjunction with metabolite (withaferin-A).

Comparative analysis of leaf and root of *Withania somnifera* ('CIM Poshita') at two different stages of plant development [(≤ 6 week-old; young) and (12-18 week-old; mature)] plants at each developmental stage: Panel A, withaferin-A contents (% DW). Panel B, Abundance of obtusifoliol-14á-demethylase (*ODM*) transcripts. Panel C, Abundance of sterol methyl transferase (*SMT1*) transcripts.



2.29. Detection of DNA polymorphism in *Papaver somniferum* genotypes differing in straw morphinan alkaloid content (O.P. Dhawan, Ashutosh Shukla, Karuna Shankar, A.K. Shasany)

Thirty-two distinct accessions of *Papaver somniferum* were screened for morphinan alkaloid content in the straw. The combined content of major morphinan alkaloids (morphine+codeine+thebaine) was found to vary in the range 0.2260–0.0683%. Two genotypes each, were selected as prototypes for low [I-48 (0.0683%) and I-344 (0.0878%)] and high [Pps-1 (0.2260%) and N-3 (0.2074%)] morphinan alkaloid content for studying DNA polymorphism. RAPD analysis of these four genotypes using 80 primers could not detect the polymorphism. However, AFLP analysis of these genotypes with 12 EcoRI/MseI primer pairs could distinctly group the high and low-morphinan alkaloid genotypes separately. Furthermore, 50 AFLP fragments, specific to high-straw morphinan alkaloid genotypes (Pps-1 and N-3) and 28 DNA fragments specific to low-straw morphinan alkaloid genotypes (I-48 and I-344) could be identified. This investigation is the first report on the polymorphism identified in the genotypes differing in their straw morphinan alkaloid content.

2.30. Optimization of efficient *in-vitro* mass multiplication protocol for Mango ginger - *Curcuma amada* (Suchitra Banerjee, Laiq-ur-Rahman)

Optimization of hormonal combinations led to rapid in vitro propagation of *Curcuma amada* through the use of young sprouts of rhizome explants. Direct regeneration of multiple shoots could be obtained on culture of the sprouted explants on Murashige and Skoog's (MS) medium containing different concentrations of TDZ. Elongation, maturation and rhizogenesis occurred on transfer of the regenerated shoots to MS medium supplemented with optimized concentrations of BAP and NAA. The differentiated complete plantlets were hardened and transferred to the glass-house with 78% survival.

2.31. Molecular characterization of different accessions of *Curcuma amada* (Suchitra Banerjee)

The RAPD based DNA fingerprinting has been carried out with four accessions of *C.amada*, collected from four different geographical locations. Overall 30 different primers were used for RAPD, and only 19 primers showed positive amplification. Dendrogram was constructed with UPGMA method. *C.amada* from Eastern I and Southern India showed 80% similarity, whereas *C.amada* from Central and Eastern India II showed 56% similarity.

Project Title: Biological & Chemical transformation of plant compounds for the production of value-added products of therapeutic /aroma values

2.32. Biotransformation of Artemisinin by *Hyoscyamus muticus* and *Valeriana wallichii* hairy root clones (Suchitra Banerjee, R.S. Bhakuni)

Experiments were conducted with pre-selected hairy root clones of two medicinally important plants,i.e, *Valeriana wallichii* and *Hyoscyamus muticus* for the biotransformation of a potent antimalarial sesquiterpene – artemisinin. *V. wallichii* hairy root clone revealed bioconversion of artemisinin to deoxyartemisinin. *H. muticus* hairy root clone biotransformed artemisinin to a new sesquiterpene in addition to its conversion to Deoxyartemisinin. Structures of the derivatives were confirmed by spectroscopic analysis which confirmed the new derivative as a ring contracted analogue of artemisinin having molecular formula $C_{15}H_{22}O_3$.

In vitro micropropagation of *Curcuma amada*

Project Title: Genotype designing for speciality crops in MAPs

2.33. A single step biphasic protocol for conservation and assessment of genetic fidelity of regenerated plants of *Rauvolfia vomitoria* –an endangered medicinal tree (A .K. Kukreja)

In the present investigation a single step, biphasic protocol has been established for the propagation and small term conservation of *Rauvolfia vomitoria* through encapsulation technique. Nodal explants from a 10 year old tree served as resource material. Explants from axillary micro cuttings of *R. vomitoria* were encapsulated in calcium alginate complex and cultured under similar conditions for short term conservation and further enhancement of number of propagules, More than 85% of encapsulated explants showed multiple shoot growth and thereafter resulted in 100% rhizogenesis within 40 days of culture. Encapsulated beads could be stored up to 180 days under moistened conditions.

2.34. Molecular docking analysisto map the binding site of squalene synthase inhibitors on dehydrosqualene synthase of *Staphylococcus aureus* (Ashok Sharma)

This work attempts to focus on squalene synthase inhibitors, lapaquistat acetate and squalestatin analogs on dehydrosqualene synthase (CrtM) enzyme of *S. aureus*. Mode of binding of lapaquistat acetate and squalestatin analogs on dehydrosqualene synthase (CrtM) enzyme of *S. aureus* was identified by performing docking analysis. Molecular docking analysis results of standard dataset (Biophosphonate derivatives) were compared with the test compounds used in the study. Based on the molecular docking analysis, it was found that the His18, Arg45, Asp48, Asp52, Tyr129, Gln165, Asn168 and Asp172 residues interacted with comparatively high frequency with the inhibitors studied. Comparative docking study confirmed the involvement of these residues of dehydrosqualene synthase enzyme with the inhibitors studied. Studies on ADMET properties might provide insights to develop new drugs to target the virulence factor, dehydrosqualene synthase of *S. aureus*.

2.35. *In vitro* and *in vivo* biological activities including drug targets and pharmacological activities (Ashok Sharma, M.P. Darokar)

With the aim of exploring the active site of FtsZ of pathogenic bacterium *Staphylococus aureus*, domain and motif study was done at the sequence level. The study investigated the functional site of *S. aureus* FtsZ by molecular docking, the known cell division inhibitors, thymol, carvacrol, berberine, cinnamaldehyde, trans-cinnamic acid as well as other known antibacterial from plants. Furthermore, the study found oenostacin to bind at site involving residues same as known cell division inhibitors.

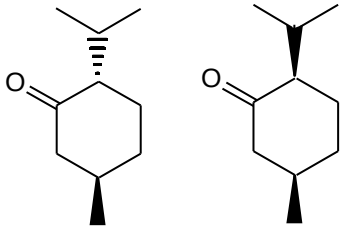
3. CHEMICAL SCIENCES

ANALYTICAL CHEMISTRY (CENTRAL INSTRUMENTATION)

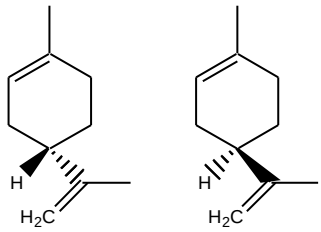
Project Title: Science and technology support towards strengthening of R&D base in medicinal and aromatic plants (C.S. Chanotiya, Anju Yadav)

3.1. Enantiomeric separation method for menthone and isomenthone stereoisomers

Enantiomeric separation method for chiral terpenoids such as (+/-)-limonene, (+/-)-menthone and (+/-)-isomenthone enantiomers has been developed for mint varieties (*Mentha piperita* and *M. arvensis*).



(-)-menthone (+)-isomenthone



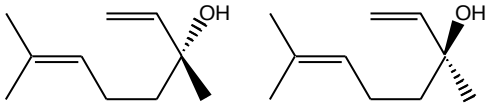
(*S*)-(-)-limonene (*R*)-(+)-limonene

3.2. Method for monitoring the emission of volatile organic compounds from flowers of *Jasminum sambac* using solid-phase micro-extraction fibers and gas chromatography with mass spectrometry detection (C.S. Chanotiya, G.C. Uniyal, Anju Yadav)

Solid-phase micro-extraction (SPME) fibers coated with PDMS (100 µm), PDMS/DVB (65 µm), Carboxen/PDMS (85 µm), DVB/Carboxen/PDMS (50/30 µm) and polyacrylate (85 µm), coupled with gas chromatography (GC-FID) and gas chromatography-quadrupole mass spectrometry (GC-MS), were used to monitor the emission pattern of biogenic volatile organic compounds (BVOCs) from living and plucked flowers of *Jasminum sambac* at different time intervals during a day. Variations in the aromatic constituents were observed with respect to time of plucking and also between live (intact) and plucked flowers.

3.3. Enantiomeric excess for (3*s*)-(+)-linalool (C.S. Chanotiya, Anju Yadav)

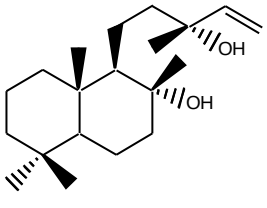
Variations and authentication of volatile constituents of *Cinnamomum tamala* leaf essential oil was characterized by enantio-GC-FID, capillary GC-FID and GC/MS. The oil samples were analyzed for two consecutive years. Linalool content varied from 6.4–8.5% to 5.7–16.2%, respectively in first and second year oil samples, while (*E*)-cinnamyl acetate was higher 10.0–22.7% in second year collection. Using a 10% heptakis (2,3-di-*O*-methyl-6-*O*-tertbutyldimethylsilyl)-â-cyclodextrin as the chiral stationary phase, optically pure (3*S*)-(+)-linalool was found in both the oil samples leading to complete enantiomeric excess for (3*S*)-(+)-enantiomer.



(3*R*)-(-)-linalool (3S)-(+)-linalool

3.4. Identification of (-)-sclareol in *Salvia sclarea* essential oils (C.S. Chanotiya, Anju Yadav, A.K. Singh)

Salvia sclarea L. samples collected from three different climatic regions viz., Kashmir, Uttarakhand and Uttar Pradesh were analyzed by enantio-GC-FID and GC/MS. Linalool (23.6%), linalyl acetate (51.2%) and sclareol (1.3%) were found in Lucknow sample while the Kashmir sample contained the highest percentage of linalyl acetate (60.8%), lowest linalool (14.5%) and sclareol (1.3%). In contrast, the oil from Purara (Uttarakhand) showed highest percentage of linalool (29.8%), and sclareol (2.3%). High enantiomeric excess was observed from 76.8-79.5% for (3*R*)-(-)-linalool and 78.8-99% for (-)- sclareol.



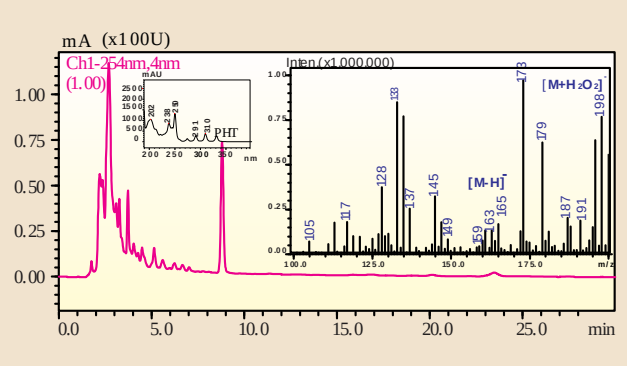
(-)-sclareol

3.5. Identification of methyl eugenol rich *Melaleuca decora* essential oil (C.S. Chanotiya, Anju Yadav, S.C. Singh)

The essential oil of *Melaleuca decora* twigs collected by hydrodistillation was analyzed by GC-FID, GC-MS and ^{1}H-, ^{13}C-NMR experiments. The most abundant compound in the twig oil is methyl eugenol (92.4%). In terms of molecular diversity, phenylpropanoids dominate *Melaleuca decora* essential oil with low terpenoid (3.9%) proportion.

3.6. HPLC method development and validation of cytotoxic agent phenyl-heptatriyne in *Bidens pilosa* with ultrasonic-assisted cloud point extraction and preconcentration (Karuna Shanker)

Extraction and pre-concentration of a bioactive marker compound, phenyl-1,3,5-heptatriyne (PHT) from *Bidens pilosa*, prior to HPLC has been demonstrated using both organic and ecofriendly non-ionic surfactants. The optimized cloud point extraction procedure has been shown to be a potentially useful methodology for the preconcentration of the target analyte. Recovery was satisfactory and the method is reliable with appreciable intraday and interday precision. The limits of detection and quantitation were 1.84 and 6.13 mg/mL, respectively. The method was validated following international guidelines and successfully applied for quantitative assays of cytotoxic compound phenyl-1,3,5-heptatriyne in *Bidens pilosa*.



Representative HPLC chromatograms: ethylacetate extract of aerial part of *Bidens pilosa* (100mg/mL). Characteristic UV and ESI-MS mass spectra of PHT are shown in the inset of chromatogram.

3.7. Simultaneous quantification of diterpenoids in *Premna integrifolia* using a validated HPTLC method (M.M. Gupta)

P. integrifolia plant extracts were separated on silica gel 60F_{254} high-performance thin layer chromatography plates using hexane/acetone/ethylacetate (60:20:20, v/v) as mobile phase. The quantitation of diterpenoids was carried out using densitometric reflection/absorption mode at 475nm after post-chromatographic derivatization using vanillin–sulfuric acid reagent. The method was validated for peak purity, precision, robustness, limit of detection (LOD) and quantitation (LOQ). The method reported here is simple and reproducible.

3.8. Chemical Investigation of *Premna integrifolia* (M.M. Gupta)

A detailed chemical investigation of different plant parts led to the isolation and characterization of number of phytochemicals belonging to the various group of compounds i.e. diterpenoids (1α, 3α, 8β-trihydroxy-pimara-15-ene, 6α, 11, 12, 16-tetrahydroxy-7-oxo-abieta-8, 11, 13-triene and 2α, 19-dihydroxy-pimara-7, 15-diene), steroid (beta-sitosterol), flavonoid (quercetin) and iridoid glycosides (10-*O-cis-p*-coumaroylcatalpol, premnosidic acid, 4"-hydroxy-E-globularinin, 10-*O-trans-p*-coumaroyl-6-O-α-L-rhamnopyranosyl catalpol)

3.9. Chemical Examination of *Vitex trifolia* (M.M. Gupta)

Detailed chemical investigation of *Vitex trifolia* led to the isolation of number of phytochemicals viz. p-hydroxy benzoic acid, p-methoxy benzoic acid, chrysoplenol-D, casticin, stigmasterol, agnuside, a lower fatty molecule, a steroid and three diterpenoids. The structural characterization of all isolated phytomolecules were performed with the help of spectroscopic (^{1}H, ^{13}C NMR, COSY, HSQC, HMBC etc.) data.

3.10. Chemical transformation of K001 into compounds having potential antipsychotic activity (S.K. Srivastava, M.M.Gupta, R.K. Verma)

Isolation and characterization of antipsychotic compound K001 from the active chloroform extract of MAP 1597 was carried out. Further K001 was chemically transformed into eight semi-synthetic derivatives. All the derivatives were evaluated for their *in-vitro* and *in-vivo* antipsychotic activity.

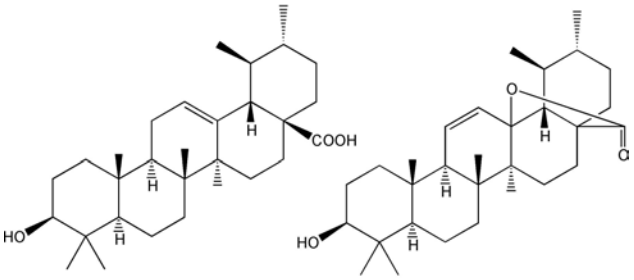
3.11. Cytotoxic evaluation of semi synthetic ester and amide derivatives of oleanolic acid (S. K. Srivastava)

Roots of *Lantana camara* L. were chemically investigated, resulted in the isolation and identification of a cytotoxic agent, oleanolic acid as a major constituent. Oleanolic acid was converted into six semi-synthetic ester and seven amide derivatives. The ester derivatives showed 3-6 times more selective activity against the human ovarian cancer cell line (IGR-OV-1), while amide derivatives showed 16-53 times more selective activity against the human lung cancer cell line (HOP-62), compared to oleanolic acid.

3.12. Preparative-scale separation of anticancer triterpenes from *Eucalyptus hybrid* by centrifugal partition chromatography (S.K. Srivastava)

Leaves of *Eucalyptus hybrid*, are a good source (~1.2 %) of anticancer triterpenes, ursolic acid and ursolic acid lactone.

Centrifugal Partition Chromatography (CPC), was successfully applied in the separation of close R_f complex anticancer triterpenes directly from a fraction of *Eucalyptus hybrid*. Using a two-phase solvent system composed of hexane/ethyl acetate/methanol/water (1:2:1.5:1 v/v/v) where 2% ammonia solution was added in lower aqueous mobile phase to achieve pH 9.5. From 1.5 g of a fraction, 145 mg of ursolic acid and 72 mg of ursolic acid lactone were obtained in 95.4 % and 94.8 % purities. Total yield recovery was >94 % and the isolated triterpenes were characterized on the basis of their ^{1}H, ^{13}C-NMR and ESI-MS data.



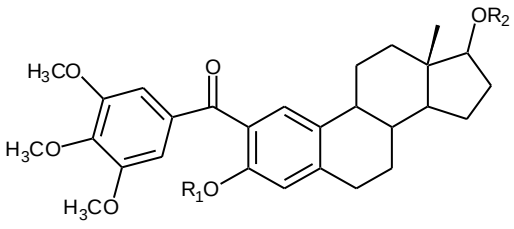
1- Ursolic acid 2- Ursolic acid lactone

3.13. Synthesis and *in-vivo* evaluation of anticancer lead molecule (A.S. Negi, Debabrata Chanda)

3-(3, 4, 5-trimethoxyphenyl)-4,5,6-trimethoxy, 1-indanone semi-synthetically prepared from gallic acid, a plant phenolic acid was found to be a potential cytotoxic agent in MTT assay. It exhibited significant anticancer activity in various *in-vivo* cancer models in mice. It cured about 55% of cancer in Swiss mice in Ehrlich Ascites Carcinoma *in vivo* model at 50mg/kg dose i.p. It was well tolerated by the Swiss albino mice up to the dose level of 1000 mg/kg body weight as a single acute oral dose.

3.14. Synthesis of phenastatin analogues on steroidal framework as anticancer agents specifically for hormone dependent breast cancers (A.S. Negi, J. Kotesh Kumar, Debabrata Chanda, Karuna Shanker, C.S. Chanotiya)

Gallic acid based phenastatin analogues were synthesized as possible anticancer agents on modification of estrone at 2-position. Some of the analogues exhibited significant anticancer activity against MCF-7 and T47D hormone dependent breast cancer cell lines. Compounds were further evaluated for *in-vivo* estrogen agonistic and estrogen antagonistic



R_1 = H/CH_3
R_2 = H/Ac

activities in female Sprague–Dawley rats. The most active analogue was evaluated for acute oral toxicity in Swiss albino mice and was found to be well tolerated up to 300mg/kg dose.

3.15. Chemo-biological standardization of *Oenothera biennis* (Karuna Shanker)

A hydrophilic interaction liquid chromatographic (HILIC) method for the quantitation of Oenotheralanosterol A and B (Oen-A & Oen-B) and gallic acid (GA) in *O. biennis* was developed using solvents with optimum extraction efficiency. Ultrasonic assisted extraction (UAE) with methanol was found to be suitable. The accuracy of the method was checked out by a recovery study and the average percentage recovery ranged between 92-98%.

3.16. Chemical modification of phytol for antitubercular activity (A.S. Negi, Dharmendra Saikia, Debabrata Chanda, J. K. Kumar, C.S. Chanotiya)

Phytol, a diterpene alcohol was modified to several analogues for antimycobacterial activity. Three of the analogues exhibited potent activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain radiorespirometrically. The most potent analogue was further evaluated for *in vivo* toxicity in Swiss albino mice and was well tolerated by the experimental animals up to 300 mg/kg body weight as a single oral acute dose.

3.17. Influence of *Moringa oleifera* on pharmacokinetic disposition of rifampicin using HPLC-PDA method: a pre-clinical study (Anirban Pal, D.U. Bawankule, M.P. Darokar, Karuna Shanker, M. M. Gupta, N.P. Yadav)

The influence of active fraction isolated from pods of an indigenous plant, *Moringa oleifera* (*MoAF*) was studied on the pharmacokinetic profile of the orally administered frontline anti-tuberculosis drug rifampicin (20 mg/kg b.w.) in Swiss albino mice. The result of this study suggested that the bioavailability-enhancing property of *MoAF* may help to lower the dosage level and shorten the treatment course of rifampicin.

3.18. Development of new & rapid diagnostic method for early detection of viruses and phytoplasma infection on MAPs (P.V. Ajayakumar, Abdul Samad, Ashutosh Shukla)

A new severe little leaf disease was observed on *Portulaca grandiflora*. Characteristic symptoms, ultrastructural studies, antibiotic response and amplification of 16S ribosomal DNA fragments (about 1.5 kb and 1.2 kb) by PCR from infected samples, suspect the involvement of phytoplasma as a pathogen. Here, the status of PLL (EF651786) is verified by virtual simulation RFLP analysis of 16S rRNA genes analysis

of the F2n/ R2 sequences of closely related strains of the 16SrVI group using 17 restriction enzymes.

3.19 Simultaneous determination of three steroidal glycoalkaloids in *Solanum xanthocarpum* by high performance thin layer chromatography (Karuna Shanker, S.K. Srivastava, and M.M. Gupta)

A new HPTLC method has been developed for the simultaneous quantitation of three bioactive steroidal glycoalkaloid (SGA) markers, solasonine (SN), solamargine (SM) and khasianine (KN) in the plant *Solanum xanthocarpum*. Extraction efficiency of targeted SGAs from plant matrix using methanol and acidified methanol were studied using percolation, ultrasonication and microwave techniques.

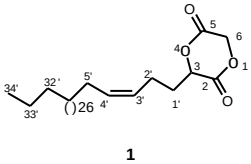
PHYTOCHEMISTRY

3.20. Chemical transformation for value addition: Transformation of withasteroids (LN Misra)

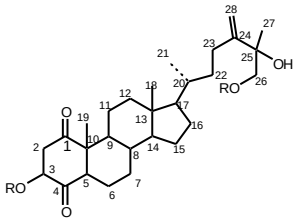
The leaves of *Withania somnifera* are extremely rich in withasteroids. Therefore, chemical modifications have been attempted to introduce value addition. The epoxide group present in the B ring has been attempted to several changes to yield hydroxyl, imine, chloro, esters etc. functional groups. The biological activity is under test.

3.21. Isolation of compounds from the Hexane extract of *Withania somnifera* leaves (LN Misra)

We have isolated 2 new compounds from the non polar extract of the leaves. One is the unusual 1,4-Dioxane derivative (1) while the other is an ergosterol derivative (2). The structure was elucidated by spectroscopic techniques, especially using the NMR spectra.



1



2, R= H
2a, R= Ac

3.22. Development of improved extraction techniques for essential oils (PK Rout)

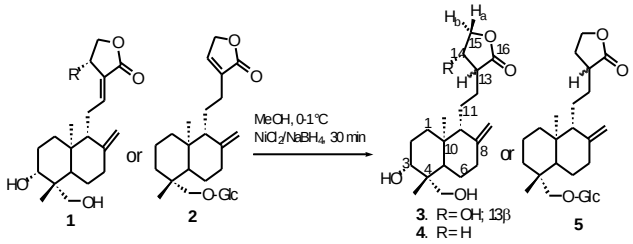
The study of different extraction processes of *Mimusops elengi*, *Murraya paniculata* and *Michelia champaca* flowers have been carried out. The liquid CO_2 extraction was found to be the most suitable process to obtain organoleptically superior floral extract as it was enriched with chief compounds with very few percentage of waxy (fatty acids and high molecular mass hydrocarbons) compounds. On the other hand, conventional solvent extraction process contained very high amounts of waxy compounds making it unsuitable for pharmaceutical and high grade perfumery application.

A new compound (Manool) was identified in *Murraya paniculata* flowers. The liquid CO_2 extract of flowers contained 30% of Manool. Manool is an oxygenated diterpenoid from the labdane class.

3.23. Pharmacophore driven synthesis using plant scaffolds (P.K. Chaudhuri)

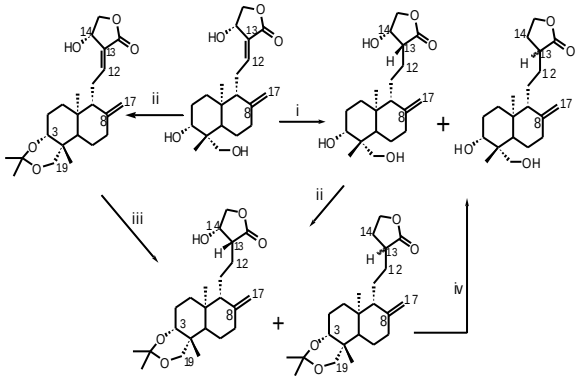
With the objective of structure determination of marker chemicals from Indian medicinal plants and structural modification of major markers as scaffold to generate new chemical space for biological and SAR study, the following progress has been made.

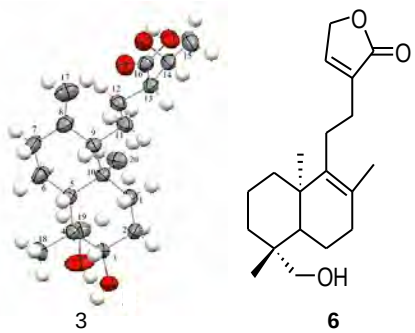
The γ-butyrolactone moiety of andrographolide on reduction of its exocyclic double bond gave 12,13R-dihydroandrographolide **3** stereoselectively and its absolute stereochemistry was confirmed by NMR and X-ray study (**3**). Neoandrographolide on reduction gave **5** without stereoselectivity under similar conditions. Both **3** and **5** lost anticancer activity due to the sp3-hybridisation of C-13 with loss of α-alkylidene function in γ-butyrolactone pharmacophore. The structure of two new scaffolds synthesised from neoandrographolide was established as **6** and **7**.



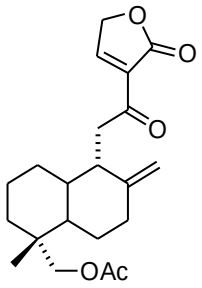
1 2 3. R=OH; 13β 4. R=H 5

A method was optimized for the extraction of bioactive saponins from *Centella asiatica* using green chemistry, and the content of asiaticoside and madecassoside is 10%.





3 6



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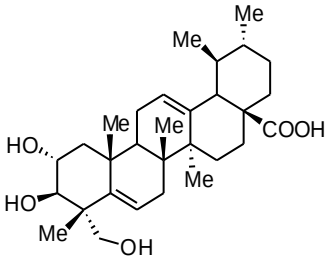


2α,3β,23 -trihydroxy-urs-5,12-diene-28-oic acid

8

The structure of a new compound from *Centella asiatica* was identified as **8.** Asiaticoside and madecassoside are useful as nerve tonic, dementia and wound healings.

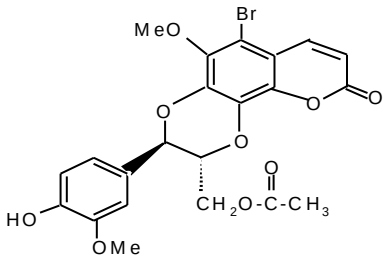
3.24. Discovery and preclinical studies of new bioactive molecules (natural and semi-synthetic) & traditional preparations: (PK Chaudhuri, S. Singh, Ram Rajasekharan)

The objective is to isolate possible anti-malarial lead bioactive molecule for structural modification. A bioactive antimalarial fraction. A compound was identified as betulinic acid and others are from steroids and phenolic class of compounds. Further identification of the compounds is in progress.

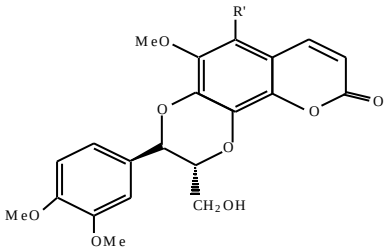
PROCESS CHEMISTRY & CHEMICAL ENGINEERING

3.25. Biological and chemical transformation of plant compounds for production of value added products of therapeutic/aroma value (S.K. Chattopadhyay, D.U. Bawankule)

Potent anti-inlammatory activity of some of the derivatives of Cleomiscosin A:



1a

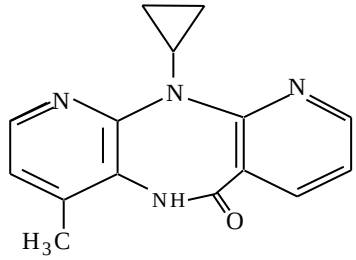


2a: R' = Cl

Different derivatives of cleomiscosin A, the major coumarino-lignoids of the seeds of *Cleome viscosa* were prepared and tested for their in-vitro anti-inflammatory activities. The compound **1a, 2a** and **3a** showed significant inhibition of pro- inflammatory targets in lipo-polysaccharide (LPS) induced inflammation in primary macrophages cell culture model when compared with the standard Diclofenac sodium.

3.26. Isolation of a minor metabolite from the seeds of *Cleome viscosa* (S.K. Chattopadhyay, Sudeep Tandon)

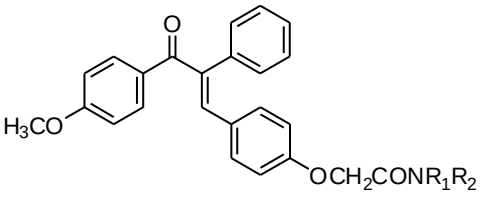
During up scaling of Cleomiscosins A, B and C from the seeds (100 kgs) of *Cleome viscosa*, a minor nitrogenous compound, $C_{15}H_{14}N_4O$, (m/z 267, $M+H^+$), was isolated. The UV (λ_{max} 325, 275 sh, 240 nm) and IR spectrum of the molecule suggested the presence of a α, β unsaturated lactam system in the molecule. ^{13}C-NMR of the compound was done. The structure of the molecule was determined unequivocally by a single crystal X- ray analysis.



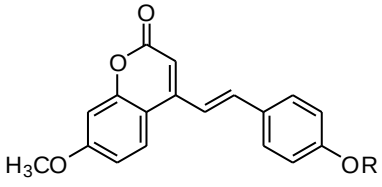
Project Title: Synthesis of bioactive molecules of therapeutic significance

3.27. Evaluation of substituted chalcones and chromanones as antitubercular agents (Atul Gupta, Debabrata Chanda, Dharmendra Saikia)

Substituted chalcones (prototype-1) and chromanones (prototype-2) were synthesized as potential antitubercular agents and were evaluated for their antitubercular efficacy in *Mycobacterium tuberculosis* strain. Five out of 17 compounds showed significant *in vitro* antitubercular activity at 30 μg-ml^{-1}. Compound ATG-1 was further studied for *in vivo* toxicological studies in Swiss albino rat model. ATG-1 is well tolerated by the Swiss albino mice up to 1000 mg/kg body weight as a single acute oral dose.



Prototype-1



Prototype-2

3.28. Processing of bioactive compounds from *Glycyrrhiza glabra* and and their bioevaluation (R.S. Bhakuni, Anirban Pal)

7,4'-Dihydroxyflavanone (DDF), isolated from the roots of *Glycyrrhiza glabra*, was derivatized to its 7, 4'-diacetate, 4'-acetate, 7, 4'-dibenzoate and isoliquiritigenin. They were evaluated for *in vitro* hepatoprotective activity against D-galactosamine lipopolysaccharide (GalN/LPS) induced toxicity. *In-vitro*-hepatotoxicity was manifested by a significant increase ($P < 0.05$) in liver toxicity biomarkers (SGPT, SGOT, ALKP, triglyceride, LPO, NO and LDH). The level of biomarkers in the treatment groups was significantly decreased ($P < 0.05$) when compared with the GalN/LPS group. The results revealed that isoliquiritigenin exhibited better hepatoprotective activity than DDF and its derivatives

Project Title: Gene bank utilization strategies: conservation to bio-prospecting genes/molecules/products.

3.29. Insect feeding deterrent and growth inhibitory activities of scopoletin, isolated from *Artemisia annua* against *Spilarctia obliqua* Walker (A.K. Tripathi, R.S. Bhakuni)

A methoxy coumarin from the stem part of *A. annua* was isolated and confirmed as scopoletin through spectral study. The compound was evaluated for its feeding deterrence and growth inhibitory potential against a noxious lepidopteran insect, *Spilarctia obliqua*. It gave FD50 value of 96.7 µg/g diet when mixed into artificial diet. In a growth inhibitory assay, scopoletin provided 116.9% growth inhibition at the highest dose of 250 µg/g diet with a GI50 value of 20.9 µg/g diet.

4. CROP PROTECTION

MICROBIAL TECHNOLOGY & ENTOMOLOGY

4.1. Identification of a new *Begomovirus* disease on *Hemidesmus indicus* (L.) R. Br. and its partial characterization (Mohd. Zaim)

Hemidesmus indicus (L.) R. Br. is affected by yellow mosaic. Studies on the etiology of the disease suggested the virus being the causal pathogen. Detection of the causal virus was done using Rolling Circle Amplification (RCA). Results suggested the genome of the causal virus to be about 5.3 Kb. with biparatite genome. The virus was neither transmitted mechanically nor by seed as other Geminivirides.

4.2. Studies on effect of poppy mosaic virus (PMV-P) infection on biosynthesis of some important poppy alkaloids (Mohd. Zaim)

The effect of poppy mosaic virus on the content of various alkaloids of opium poppy was studied in four cultivars. A positive co-relation for morphine, codeine, narcotine and thebaine was observed in IM and Sampada whereas negative co-relation for papaverine existed, if infection prevails for longer time. Shweta was found to have a positive co-relation for morphine, codeine, narcotine and thebaine alkaloids whereas negative co-relation for papaverine existed, if infection prevails for shorter duration.

4.3. *Ocimum sanctum* extracts extend lifespan of *Caenorhabditis elegans* (Rakesh Pandey)

The alcoholic and hydro alcoholic plant extracts of *Ocimum sanctum* significantly modulated the lifespan of the nematode as compared with untreated control in a concentration dependent manner. At lower doses (0.01mg/ml and 0.001mg/ml) highest increase in the mean and maximum lifespan was noticed. A significant enlargement in the body size (length/width) was also observed in worms treated with aqueous and hydroalcoholic extracts.

4.4. Toxicity of nanomaterials on *Caenorhabditis elegans* (Rakesh Pandey, K.C.Gupta)

In the present experiment the toxicity of nanoparticles especially TiO_2 and ZnO have been determined. In our results it was found that smaller particles of nanomaterials were more toxic than larger particles; particles of >25nm of TiO_2 and ZnO showed LC_{50} of 77mg/L and 0.32mg/Lrespectively, whereas in case of particles of >100nm TiO_2 were nontoxic and for ZnO the LC_{50} value was 2mg/L.

4.5. Enhanced tolerance of menthol mint against root-knot nematode through mutualistic endophytes and PGPRs (Rakesh Pandey, Alok Kalra, H.N. Singh)

Mentha arvensis cv. kosi is highly infested with the nematode *Meloidogyne incognita* (Kofoid and White) resulting in severe oil yield loss. The mutualistic endophytic fungi (*Trichoderma harzianum* strain Thu, *Glomus intraradices*) and plant growth promoting rhizobacteria (*Bacillus megaterium* and *Pseudomonas fluorescens*) were assessed individually and in combination for their effects on plant biomass, oil yield, reproduction potential and population development of *M. incognita* under glasshouse conditions. Plant biomass and oil yield were enhanced both with and without *M. incognita* inoculation.

4.6. Endophytes of *Bacopa* and *Centella* and their yield enhancing potential (Alok Kalra)

Sixteen endophytic microbes were isolated from *Bacopa monnieri* and were tested in pots for their capabilities to improve growth and content of bacosides. BAC 1 and BACR 10 improved the leaf stem ratio compared to plants not inoculated with endophyte. Of the sixteen strains, a significant increase in the content of bacosides was noticed with strains BACR 4, BACR10, BACR 11 and BACR 12 (1.16 – 1.22%) as compared to uninoculated control (1.04%).

Experiments conducted with endophytes isolated from *Centella asiatica* revealed that strains CENL2, CENL 10, CENL12 and CENL 15 improved the growth of the plants (an increase of 34.98 to 63.74% over uninoculated control). An increase in leaf: stem ratio was observed with strains CENL3, CENL4 and CENL 7. An increase in the content of asiaticosides was observed in plants inoculated with CENL2, CENL7, CENL 9 and CENL 17.

4.7. Consortium of AM fungi and IAA producing, and phosphate solubilising bacteria (Alok Kalra)

Experiments were conducted to establish a consortium of microbes involving AM fungi and bacteria with abilities to produce IAA (IAAP) and solubilise phosphates (PSB) effective in improving yields in *Bacopa monnieri.* Of the AMF, *Glomus mosseae* (Gm) was found to be the most effective in improving total weight of the plant. Of the bacteria, PT17 and B1 were also effective in improving herb yields. Both these bacteria along with Gm significantly enhanced the herb yield compared to when inoculated alone. Gm+ PT 17 and Gm + B1 enhanced the yields by 35 and 30% and the content of bacoside-A increased by 39.6 and 39.0% respectively over control.

Interaction of some endophytes (found superior in our earlier studies) and AM fungi was also studied in *Bacopa monnieri.* A synergistic interaction was observed between Gm +BACR 10 and Gi + BACR 10, improving the total dry biomass of the plant by more than 100%.

4.8. Field evaluation of microbial consortium (Alok Kalra)

Microbial consortia of AM fungi (Gm) and a nitrogen fixing bacteria (Az 26) found superior in improving growth and content of artemisinin in *Artemisia annua* in earlier glasshouse experiments was field tested on *A. annua* var. CIM Arogya. The consortia of Gm + Az26 improved the plant height (4.9%); number of branches (55%); total shoot weight (63%); leaf dry weight (48%) and content of artimisinin (16.9%) over uninoculated control.

4.9. Leads in plant Biopesticides for pre and post harvest technology (Dwijendra Singh)

Among various plant extracts evaluated for insect growth regulator (IGR) activities, the acetone and water extract of DS/NA-Ent/01 and DS/NA-Ent/07 showed strong sub lethal effects against *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae). We have found reduction in mean larval weight of *H. armigera* ranged from 90-92% in methanol and acetone extract of DS/NA-Ent/01. The methanol and acetone extract of DS/NA-Ent/01 and DS/NA-Ent/07 showed 100% IGR activity at 2% concentration against *H. armigera* during reporting period. We have found that aqueous extract of DS/NA-Ent/01 and DS/NA-Ent/07 reduced the mean larval weight ranging from 44-84% as compared to control. However, aqueous extract of DS/NA-Ent/01 and DS/NA-Ent/07 showed 80-100% mean IGR activity at 2% concentration in insect diet against *H. armigera.*

PLANT PATHOLOGY

4.10. First report of pod rot on senna (*Cassia angustifolia*) in India (Mansoor Alam)

The incidence of *Bipolaris* pod rot disease on senna has been recorded for the first time in India. The disease initially appears in the form of brown, circular to irregular, minute to large necrotic spots on the pods during the month of May, 2010 which later turn into pod rot. In advanced stage of infection, disease also appears on the leaves and stem. The infected pods have the malformed seeds which lost germination capability.

(a). A typical symptom of pod rot disease on senna (b) Conidia of *B. australiensis* born on sympodial geniculate conidiophore

Isolation carried out from the infected pod on PDA invariably yielded a species of *Bipolaris*. Conidiophores are brown, simple or branched, geniculate and sympodial bearing conidia. Conidia varying in size 20-30 µm x 7.5-10 µm (av.22.9 x 9.9 ìm), 3 septate, fusoid to cylindrical, light brown and germinate through bipolar ends. Based on cultural and morphological characters the fungus was identified as *Bipolaris australiensis*. The pathogenicity was established on healthy pods by artificial inoculation under glasshouse condtion.

4.11. First report of *Colletotrichum kahawae* causing anthracnose disease of asparagus in India (Mansoor Alam, H.N. Singh)

The incidence of anthracnose disease was first noticed in the months of July-August, 2010 in the field of

asparagus at CIMAP, Lucknow and its adjoining areas in northern India. The isolations from the infected plant parts on PDA invariably produced a species of *Colletotrichum*. Based on cultural and morphological characters the fungus was identified as *Colletotrichum kahawae*. The identification was later confirmed by IMTECH, Chandigarh, India and culture was deposited there (Accession no. MTCC-10183). This is the first report of *C. kahawae* causing anthracnose disease on asparagus in India.

4.12. Occurrence of *Synchytrium endobioticum* on kalmegh in India (Mansoor Alam, Abdul Samad, H.N. Singh)

An outbreak of *S. endobioticum* infection on kalmegh

Typical anthracnose symptoms produced by *C. kahawae* on asparagus

(*Andrographis paniculata*) was noticed in the experimental fields at CIMAP, Lucknow and its adjoining areas. The disease initially appears in the form of warty cauliflower-like outgrowths over the surface of the petiole and midrib of the infected leaves and stem, which later turn into dark brown and resulting into stunting of the growth of the infected plants. The causal organism was identified as *S. endobioticum* on the basis of its typical morphological characters. Though it is very common on potato, its occurrence on kalmegh is the first report from India.

4.13. Occurrence of a new phytoplasma disease on Ajwain (*Trachyspermum ammi*) (Abdul Samad, P.V. Ajayakumar, A.K. Shukla)

A new phytoplasma disease on *Trachyspermum ammi*, commonly known as Ajwain was observed in northern India. The symptoms included small chlorotic leaves, highly proliferating shoots, witches' broom appearance, shortened internodes and an overall stunted growth. Poor flower heads and fruit setting caused considerable yield losses to the crop. Pleomorphic bodies were detected by TEM in the phloem cells of diseased plants. The disease etiology was investigated by direct and nested-PCR using phytoplasma-specific primers, DNA sequencing, and phylogenetic analysis. This is the first report of phytoplasma infection on Ajwain.

5. GENETICS AND PLANT BREEDING

PLANT BREEDING

Project Title: Genetic enhancement of obligate asexual MAPs

5.1 Genetic enhancement of aromatic grasses, *Cymbopogons* through genomic and genetic manipulation

5.1.1. Genomic manipulation in *Cymbopogons* (U.C. Lavania)

The raw autotetraploid clone developed for the Lemongrass (*C. khasianus*) clone "Suwarna" has been screened for cytological purity and progressive polyploid stability.

5.1.2. Polyploidy breeding in *Cymbopogons* (U.C. Lavania and R.K. Lal)

The cytologically pure and genetically stable clones developed in Lemongrass (*Cymbopogon flexuosus*), Citronella (*C. winterianus*), Palmarosa (*C. martinii*), and geraniol rich species of *Cymbopogon*, namely *C. nardus* (clone CN-5), *C. jwaruncusha x confertiflorus*, all with 2n=20, 4x=40, and *Cymbopogon khasianus* x *pendulus* – clone CKP-25 (2n=60, 4x=120) were evaluated in initial and advance evaluation experiments in randomized block design, in three replications, plot size = 8 M^2 (30 x 30 cm plant to row spacing), over three locations. The mean performance over locations is given in the Table.

Observations recorded show that the induced tetraploid of *C. flexuosus*, clone 'Krishna' and clone 'Pragati', auto-tetraploids of *C. winterianus* clone Bio-13, and CIM-Jeeva, and the auto-tetraploids of geraniol type *Cymbopogon* species, *C. nardus*, *C. jwaruncusha x confertiflorus*, and *C. winterianus* exhibit superiority for fresh herb, oil yield and oil content.

Mean performance of various induced auto-tetraploid clones in aromatic grasses

SN	Entries	Herb yield (kg/plot)	Oil content (%)	Oil yield (g/plot)
A.	**Lemongrass type**			
1.	Autotetraploid (4x) of clone "Krishna"	13.00	0.915	119.60
2.	Autotetraploid (4x) of clone "Pragati" (4n)	18.63	0.63	117.37
3.	Autotetraploid (4x) of clone "CKP-25"	8.50	0.64	54.50
4.	Check "Neema"	10.29	0.65	67.62
B.	**Java citronella**			
1.	Autotetraploid (4x) of clone "Bio 13"	10.65	1.035	110.905
2.	Autotetraploid (4x) of clone "CIM-Jeeva"	8.485	1.125	94.422
3.	Check- clone "Manjusha"	6.913	1.07	75.92
C.	***Cymbopogon* spp. (geraniol type)**			
1.	Autotetraploid (4x) of clone "CN-5"	52.83	0.46	120.78
2.	Autotetraploid (4x) of clone "Jamarosa"	26.75	0.52	137.30
3.	Autotetraploid (4x) of Palmarosa	25.63	0.56	138.30
4.	Check- "PRC-1"	23.65	0.42	99.33

5.2. Breeding for genotype designing in Asparagus, Vetiver and Curcuma

5.2.1. Development of high yielding varieties of khus (*Vetiveria zizanoides*) (Ashok K. Singh)

Ten superior clones with oil content above 2.2% in replicated trial, developed out of polycross progenies were under evaluation for their performance with respect to fresh root yield and oil content again. Fresh root yield and oil content ranged from 0.50 – 70.7g/ plant and 2.2 – 2.96 percent, respectively against check (Gulabi-1.76, Kesari-1.04, Dharni-1.92, KS1-2.03%). Thirty half sib progenies have been raised through seeds for evaluation.

5.2.2. Development of high yielding variety of Satavar (*Asparagus racemosus*) (Ashok K. Singh)

Mutation breeding approach: M_2 generation in Satavar (seeds treated at 10, 20, 30 and 40 KR) are under evaluation for desired trait. Population improvement through evaluation of half sib families. Five groups consisting of thick, thick and short, thick with medium length, long and short were made and planted in isolation for seed production and evaluation.

5.2.3. Characterization in *Curcuma longa* (Ashok K. Singh)

Sixty three clolines of Curcuma were characterized for number of shoots (1.0-4.66), plant height (71.33-178.88cm), number of leaves (6.66-10.0), length of petiole (9.00-62.66cm), length of lamina (32.33-77.50cm), width of lamina (9.99-19.94cm), leaf area (302.68-684.10cm^2), fresh tuber yield (47.45-1040.87g/ plant), colour coding of the tuber (fresh 2A-25C and dry 18A-28B) and curcumin content (0.969-3.174%) on the basis of replicated trial.

5.3. Breeding strategies for newer opportunity medicinal and aromatic plants: *Stevia*, *Centella* and Ashwagandha

5.3.1. Genetic improvement of *Stevia* and development of an improved variety for commercial cultivation (R.K. Lal)

Elite plants of Stevia *(Stevia rebaudiana)* were selected on the basis of their performance in different field evaluation trials for high herb type and chemical constituents of dry leaf powder for high steveoside, rebaudioside with low dulcoside -A content (%). Out of them genotype 3-3 was found to be highly promising for high fresh and dry leaf yield with high steveoside and rebaudioside content with low dulcoside –A content. High content of rebaudioside is responsible for less pungent stevia leaves/ powder. The variety CIMAP M of stevia with high dry herb (leaves - 43 v/s26 q/ha), high Stevioside content (12.57 v/s 3.63%), high Rebaudioside content (5.80 v/s 3.32%) and low dulcoside A content (0.20 v/s 3.69%) has been developed through half sib family selection followed by cloline breeding approach and released for commercial cultivation.

Stevia variety CIMAP-Madhu

5.4. Breeding strategies for newer opportunity MAPs: *Andrographis*, *Tinospora* and *Bacopa*

5.4.1. Performance evaluation of Genetic variants in Kalmegh (*Andrographis paniculata*) (H.O. Misra)

Thirty accessions collected from different locations in India, as also mutant progenies were raised at CIMAP experimental farm, Lucknow. The mean performance of desired accessions for economic traits including chemical constituents is given in the Table.

Entry No.	Dry Biomass wt. (g/pl.)	Leaf/ stem ratio	Plant height (cm)	Andro-graph-olide (%)	Neo-andro-graph-olide (%)
AP-25 (Nagpur)	36.63	0.860	68.75	2.872	0.559
AP-27 (Hyderabad)	26.80	0.768	63.10	2.662	0.556
AP-89 (Lucknow)	56.16	0.718	70.70	2.619	0.599
AP-20 (Jaunpur)	41.02	0.623	64.60	2.887	0.564
AP-12 (Barwani)	45.73	0.884	70.30	2.520	0.493
AP-5 (Lucknow)	46.15	1.040	69.80	2.347	0.523
AP-3 (CIM-Megha)	46.85	0.883	74.40	1.919	0.366
Range	11.32-86.37	0.513-1.418	45.7-75.3	0.941-2.887	0.063-0.599
Mean	35.43	0.860	64.73	2.218	0.438

5.5. Molecular taxonomy and basic studies in MAPs

5.5.1. Molecular characterization, ITS variation across ploidy variation in Cymbopogon (U.C. Lavania)

Amplified products of ITS1 and ITS2 regions in the diploids and their corresponding clonal autotetraploids in Cymbopogon examined for their DNA sequence complexity reveal nucleotide changes, mainly deletion to the order of 0.9. to 3.0% specific to the species with autopolyploid stabilization.

5.5.2. *In situ* chromosomal localization of 5mc methylation in Cymbopogon (U.C. Lavania)

Using 5mc mouse antibody, *in situ* immunolocalization of DNA methylation patterns has been discerned on the somatic chromosomes of Cymbopogon spp. A careful examination of DNA methylation on chromosomes underpin enhanced methylation in the tetraploids compared to their progenitor tetraploids. The observations are first of its kind and have far reaching implications in understanding molecular mechanisms of autopolyploid stability.

5.6: Breeding and characterization of Mentha species

5.6.1. Identification of high yielding clone in *Mentha arvensis* (J.R. Bahl)

A high oil yielding (128 kg/ha) menthol mint clone MBB-1022 has been identified in Initial Evaluation Trial with oil content 0.8% having 78 – 80 % menthol in the off season crop. The clone MBB-1022 is suitable for growing in late kharif season after the harvest of main crop.

5.6.2. Breeding approaches to *Mentha* spp.

a) Seventy five genetic stocks of six mint species were maintained and multiplied for use in research programmes (J.R. Bahl)

b) Seedlings of 52 nos. of half sib seed progenies of *M. spicata* were raised for their proper establishment and multiplied, and data recorded for morphometric trials for their characterization and application for breeding purpose (V.R. Singh)

c) Genetic improvement in *Mentha piperita*. Fifty half sib seed progenies were raised from different seed producing stocks of *Mentha piperita* for assessment of yield potential. Out of 50, only 8 seed progenies showed high oil yield potential than their seed parents at first instance. The chemical profiles of promising lines are in progress. The strain MPH-1, christened as menthofuran mint variety CIMAP-Patra, showing oil yield potential of about 60-65 kg/ha with menthofuran content up to the tune of **46%** in its oil, has been released and licensed to AM Todd Company Limited (Birendra Kumar)

5.7. Breeding and characterization of opium poppy

5.7.1. Identification and inheritance of homeotic gene mutants in opium poppy (O.P. Dhawan)

Homeotic gene mutants developed due to deviations in flower development leading to altered floral organs like complete conversion of petals into sepals (SP-1), stamens into petals (PS-1) and partial conversion of sepals into petals (Pps-1) have been identified in our germplasm accessions. Segregation patterns of phenotypic characters in F1 and F2 were also studied.

5.8. Nuclear planting material (J.R. Bahl)

Nuclear planting material of twenty six varieties of mints, four varieties of geranium and one variety each of patchouli and silybum were multiplied and maintained in experimental field plots for use in various research projects.

5.9. Mutation breeding in Milk thistle (*Silybum marianum*) (H.O. Misra)

The major biologically active compound of milk thistle is silbinin, silymarin which is hepatoprotective, antioxidant and also have anticancer effect and procured from seeds. The mean performance of desirable mutants (M4) for economic traits have been studied.

The high seed yielding mutants were SMM-137, SMM-74, SMM-136, SMM-7 and SMM-77. Early flowering mutants were SMM-74, SMM-136, SMM-137 and dwarf mutant SMM-118.

5.10. Development of improved variety in opium poppy, *Papaver somniferum* (R.K. Lal)

Elite lines of opium poppy were selected on the basis of their performance in different field evaluation trials. Variety CIMAP AJAY was found to be highly promising for high straw and seed yield and with high alkaloids content. CIMAP AJAY is superior in straw yield (8.50 q/ha v/s check 6.80 q/ha); seed yield (9.50 q/ha v/s check 8.00 q/ha) with high percent morphine content (1.02 v/s 0.51); thebaine content (0.21 v/s 0.14) and narcotine content (0.62 v/s 0.506). The variety CIMAP AJAY of opium poppy has been developed by CIMAP through recombination breeding approach.

Variety 'CIMAP AJAY" of opium poppy

5.11. Genetic improvement of *Silybum* using classical breeding (A.K. Gupta)

The genetic improvement of *Silybum* for dwarf plant with high silymarin content was started using the induced mutagenesis. The desired selected variants were subjected to classical population improvement (gene enrichment cycle) programme. Populations of dwarf lines (up to 120 cm) with silymarin content of more than 8% are being evaluated for seed yield.

5.12. Mutation breeding in Bishop's weed (*Ammi visnaga*) (H.O.Misra)

More than 15 desirable mutant progenies were raised and morphometric observations were recorded. Seeds of genotypes related with stem colour, hairiness and deviant in umbel colour were selfed and individual plant seeds were collected. Apart from this, desirable accession seeds were irradiated through gamma rays up to 80kR and raised in field and M1 seeds were collected to raise M2 generation. Survival of plants was up to 80kR.

5.13. Genetic improvement in Sowa (*Anethum sowa*) (H.O.Misra)

More than 20 accessions collected from different places of India along with mutants were raised in replicated field trial and morphometric observations were recorded. Apart from this, 4 desirable accession seeds were irradiated through gamma rays at 10, 20, 40, 60, 80kR and raised in field. The survival of plants was only up to 40kR and M1 seeds were collected to raise M2 generation.

5.14. Genetic improvement of Pyrethrum (R.K. Lal)

Induced variability for synchronous flowering in pyrethrum is successfully induced using gamma ray irradiation. The outperforming 18 elite mutants + one check in BST placed in PST (RBD, Reps= 3, Plot size= 9 m^2). Entry No. five produced highest dry yield (1390.00 g/plot) among all followed by N0. 7 (950.33 g/plot) v/s check (192.44 g/plot) only.

5.15. Characterization and Evaluation of Ginger (*Zingiber officinale* Rosc.) (V.R. Singh)

Ginger germplasms were collected from Peruvannamuzhi (Calicut) Ambalavayal (Waynad) and local area of Thiruvananthpuram in Kerala. A total of 11 diverse germplasms were vegetatively multiplied and maintained at CSIR-CIMAP Research Farm. Morphological data of above said germplasm were recorded. Accession number ZO-09 had the highest ranking rhizome yield, primary rhizome and number of tillers/plant.

Project Title: Development of a set of true breeding homeotic mutant lines for the study of flower organ identity genes in opium poppy

5.16. Study of flower organ identity genesin opium poppy (O.P. Dhawan)

True breeding inbred lines of three homeotic mutants with partially petaloid sepals (PPS), sepaloid petals (SP) and petaloid stamens (PS) have been developed in opium poppy which are being utilized for the identification of flower organ identity genes. PPS-1 mutant is also found to be highly resistant to downy mildew (DM) caused by *Peronospora arborescens*. Different generations of the crosses are being studied to understand genetic behaviour of genes responsible for all these homeotic traits.

Project Title: Programme on malaria using medicinal and aromatic plants

5.17. Initiation of integrated program on malaria using medicinal and aromatic plants

As a first step 20 plants which have been reported in literature for anti-malarial activity and are available at CIMAP have been selected for bioactivity testing.

In another study, population improvement in *Artemisia* is being carried out for increasing artemisinin content in plants using classical breeding approach. This resulted in development of *Artemisia* populations with more than 1.2% artemisinin content. These lines are being evaluated for herb yield and other traits.

GENETIC RESOURCES MANAGEMENT

5.18. Assessment of variability based on phenotypic traits in germplasm accessions of *Papaver somniferum* L. (A.K. Gupta, Dharmendra Saikia)

A set of about 600 germplasm accessions of *Papaver somniferum* is being conserved at CIMAP. Out of above set, 108 germplasm accessions were selected and characterized for five morphometric traits namely plant height (cm), number of capsules per plant, peduncle length (cm), number of stigmatic rays on the capsule and susceptibility to downey mildew. The morphological variability for qualitative traits (Flower type and colour, Leaf margin, Type and arrangement of stigmatic rays) was documented photographically.

5.19. Character associationship and role of various traits in determining the number of stigmatic rays on the capsules of different genotypes of Papaver (A.K.Gupta)

Correlation study revealed a positive association of peduncle length with plant height (0.42) and number of stigmatic rays on the capsule (0.21). Whereas, number of capsules per plant were negatively correlated with plant height (-0.48), peduncle length (-0.24) and number of stigmatic rays on the capsule (-0.39). The number of capsules per plant exerts maximum negative direct effect (-0.39) on the number of stigmatic rays on the capsule. The positive direct effect was shown by peduncle length (0.21) and plant height (0.11). Susceptibility to Downey mildew showed a negligible negative direct effect (-0.02) on number of stigmatic rays on the capsule.

Estimates of direct (bold figures on the diagonal) and indirect effects for five morphometric traits in germplasm accessions of *Papaver somniferum*

Characters	Plant height	Capsules /plant	Length of peduncle	Downey mildew susceptibility	rg (genotypic correlation)
Plant height	**0.113**	-0.044	-0.084	0.004	-0.018
Capsules / plant	-0.054	**-0.389**	0.090	-0.008	-0.361
Length of peduncle	0.047	0.093	**0.214**	0.005	0.358
Downey mildew susceptibility	0.013	0.022	-0.013	**-0.019**	0.003

Residual Factor = 0.68

5.20. Stability of morphine content in six selected germplasm accessions of Papaver (A.K. Gupta)

Six selected germplasm accessions of *Papaver somniferum* (AP 7, AF 669, AP 616, AP 13, AP 4 and AP 10) were studied for stability of morphine content over a period of three years. On the basis of pooled results from three year data, AMMI analysis identified the AP 7 as best performing with widest adaptability (most stable) genotype. On the basis of performance and adaptability the genotypes could be arranged as AP 7>AP 13>AF 669> AP 4>AP 616>AP 10; in descending order.

5.21. Standardization of seed germination parameter in Kalmegh (*Andrographis paniculata*) and Senna (*Cassia angustifolia*) (Birendra Kumar)

Seed germination percent and germination energy (%) of Kalmegh (*Andrographis paniculata* Wall. ex Nees) variety 'CIM-Megha' at 15°C, 20°C, 25°C, 30°C, 35°C and 40°C temperatures coupled with 16h light and 8h dark photo period were evaluated. Maximum germination percentage and germination energy (%) are recorded at '25°C' (94.6, 23.6) followed by '20°C' (89.9, 22.5), '30°C' (87.5, 21.9), '35°C' (79.5, 19.9) and '15°C' (53.7, 13.4) temperatures. At 40°C seeds did not germinate. The study revealed that evaluations of germination should be carried out between 5th and 9th days after seed sowing under temperature regimes 20, 25, 30 and 35°C. Similarly in case of Senna, the maximum percentage of germination (90-95) and germination energy (22.5-23.75) were observed at 20-30 °C with 3rd day as first and 5th day as final count day.

5.22. Molecular Cataloguing of Core Collections in Papaver germplasm (Tripta Jhang, A.K. Gupta)

A preliminary screening of genetic diversity in a set of 96 accessions out of ~ 600 opium poppy accessions was done to develop molecular catalogue of core collections in *Papaver somniferum* using 15 ISSR primers. DNA was subjected to PCR amplification and 90 scorable fragments was obtained out of which 81 bands were polymorphic.

5.23. Conservation, evaluation and cataloguing of selected high value MAPs (*Gymnema sylvestris, Valleriana wallichi, Withania somnifera, Catharanthus roseus*)

5.23.1. *Gymnema sylvestris* (Tripta Jhang, A.K. Kukreja, S.S. Dhawan, R.V. Sreedhar)

To conserve and evaluate the genetic and metabolic diversity of South Indian *Gymnema sylvestre*, 16 Samples from Karnataka and Tamil Nadu and 11 accessions from North India have been collected and are being maintained under field conditions.

5.23.2. *Valeriana wallichi* (Rajesh Verma, R.S. Verma, Amit Chauhan)

Twenty one samples of *Valeriana wallichi* were collected from 21 locations of Bageshwar, Chamoli, Nainital and Almora districts of Uttarakhand. These were established and ex situ conserved at Pantnagar/Purara Resource Center. On chemical charecterization, valpotriate oil ranged from 21-46%.

5.23.3. *Withania somnifera* (A.K. Gupta, Tripta Jhang, M.M. Gupta, R.K. Verma, S.C. Singh, G.D. Bagchi)

Genetic divergence of 75 collections of Ashwagandha (*Withania somnifera*) for 10 characters was estimated. There was sustained variation in clusters distance. On the basis of relative character contribution towards genetic divergence, the highest contributing character was 12-deoxywithastramonalide content in roots (ranks 1st with 28.48 % contribution) followed by 12-deoxywithastramonalide content in leaves (ranks 2nd with 18.63 % contribution) and Withaferin-A in roots (rank 3rd with 17.82 % contribution).

5.23.4. *Catharanthus roseus* (R.N. Kulkarni, Tripta Jhang, Karuna Shanker)

A natural population of 70 genotypes comprising released varieties (3), breeding lines (30) and mutants is sown in the current year. Unigene derived microsatellite markers resource in Catharanthus is under development.

5.24. Optimization of seed rate for commercial cultivation of seed propagated Palmarosa (*Cymbopogon martinii*) crops (Birendra Kumar)

Germination percentage and germination energy (%) was evaluated at 15°C, 20°C, 25°C, 30°C and 35°C and found that the maximum germination (%) and germination energy (%) were recorded in PRC-1 (68.50, 17.12) followed by 'Tripta' (59.25, 14.81), 'Trishna' (58.25, 14.56) and 'Vaishnavi' (35.50, 8.87) at 25°C temperature. Results showed that day 2 and day 5 ('PRC-1' & 'Trishna') – 6 ('Tripta' & 'Vaishnavi') was the best for first count and final count at 25°C temperature. For evaluation of germination potential in Palmarosa seeds, 15°C and 20°C temperatures were found not suitable. On the basis of monthly germination testing conducted at 25°C temperature, stored at room temperature the germination potential of seed gradually increased up to the 6 months and thereafter showing reduction in germination potential.

6. PLANT BIOLOGY

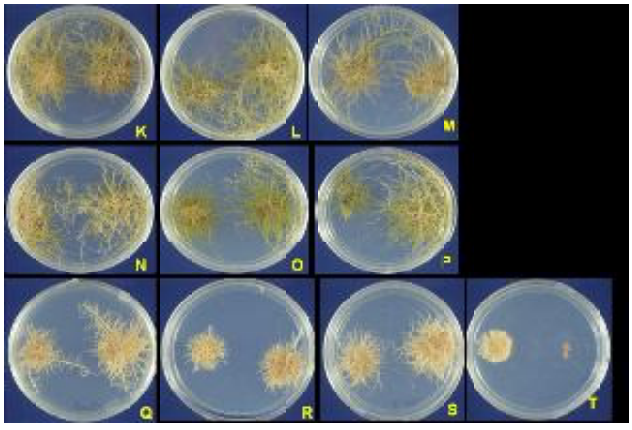
METABOLIC AND STRUCTURAL BIOLOGY

6.1. Biotransformation of withanolides by cell suspension cultures of *Withania somnifera* (N.S. Sangwan)

The biotransformation potential of cell suspension cultures regenerated from *Withania somnifera* leaf was investigated, using withanolides, viz withanolide-A, withaferin-A, and withanone as precursor substrates and they showed inter-conversion of withanolides, as well as to some unknown compounds, and released to the culture media. The bio-catalyzed withanolide formed was detected and quantified by TLC and HPLC. There appears to be substitution of 20-OH group to 17-OH in withanolide A.

6.2. Development of biosynthetically active and high frequency multiple shoot proliferation from axillary shoots and direct rhizogenesis induced root morphotypes of *Centella asiatica* (N.S. Sangwan)

A protocol has been developed for the generation of biosynthetically active and high frequency multiple shoot regeneration from *Centella asiatica*. Rhizogenesis of these under different hormonal conditions resulted in different morphotypes ranging from long green root type to the short bushy and white type. Combinations of BAP



and kinetin were able to produce highest number of multiple shoots in one month growth period and also produced asiaticoside, the medicinally important phytochemical. Incorporation of ^{14}C labeled sucrose into the multiple shoots indicate that they were biogenetically active.

6.3. Cloning of glycosyltransferase gene involved in secondary metabolism from *Catharanthus roseus and Gymnema sylvestre* (N.S. Sangwan)

Glycosyltransferase gene was isolated and cloned from *Gymnema sylvestre.* Two partial GT sequences from *Catharanthus roseus* have also been cloned and all these sequences show homology to the secondary metabolic GTs from plants.

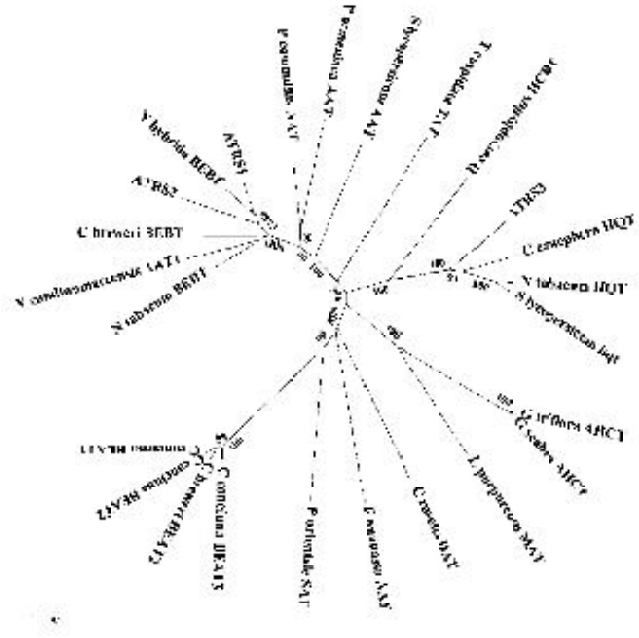
6.4. Cloning and Characterization of deoxyxylulose-5-phosphate synthase (dxs) gene from Ashwagandha (*Withania somnifera*) (R.S.Sangwan and N.S.Sangwan)

Deoxyxylulose-5-phosphate synthase (dxs) gene involved in the early steps of isoprenogenesis via non-mevalonate (DOXP) pathway has been cloned from leaf of Ashwagandha (*Withania somnifera*) experimental line, NMITLI-Hybrid into expression vector in *E. coli*.

6.5. Identification and cloning of AAT genes of BAHD superfamily in *Artemisia annua* differentially expressed in leaf, stem and root (R.S.Sangwan)

Alcohol acyltransferases (AAT) constitute a gene superfamily called BAHD and play diversified metabolic roles including synthesis of several secondary metabolites. ESTscan of transcriptome from *A. annua* glandular trichomes yielded in all 43 contigs homologous to BAHDs; filtering led to identification of at least 16 discrete BAHD members in the tissue. RT-PCR analysis confirmed the expression of the unigenes and novel transcripts. Three full length acyltransferases (AATs) were assembled and validated to be existent *in vivo*.

Phylogenetic relationship of *Artemisia annua* AATs (ATRS1, ATRS2 and ATRS3) with other members of BAHD family.



Project Title: Biological and chemical transformation of plant compounds for production of value added products of therapeutic/ aroma value

6.7. Development of QSAR models for immuno-modulatory activity of ursolic acid and other triterpenoid derivatives (Feroz Khan, Abha Meena, D. Chanda, D.U. Bawankule, S. K. Srivastava, S.K. Chattopadhyay)

QSAR studies indicated that connectivity index (order 2), sec. amine group count and ether group count correlates well with biological activity. Results indicate that ursolic acid has immunomodulatory activity comparable to bowellic acid and cichoric acid but less active than levamisol. Moreover, through molecular docking ursolic acid showed high binding affinity for immunomodulatory receptors such as, Cyclo-oxygenase-2, Human NF-kappaB p52, Tumor necrosis factor (TNF-alpha), Viral Interleukin-10, and Phosphatidyl inositol 3-Kinase Regulatory alpha subunit (PDB: 2IUG), thus considered active triterpenoid compound. QSAR model equation:

6.6. Volatile monoterpene oil biosynthesis in rose-scented geranium (*Pelargonium* spp.) (R.S. Sangwan and N.S. Sangwan)

Monoterpene biosynthesis in rose scented geranium has been shown to involve non-mevalonate pathway of isoprenogenesis. However, none of the pathway steps have been characterized. We have cloned deoxyxylulose-5-phosphate synthase (*dxs*) gene from its leaves, *dxs* being the committed and regulatory step in the pathway. The 2,801 bp clone comprises a 2157 bp ORF and 5' and 3' UTRs.

Comparison of experimental and predicted *in-vivo* activity data calculated through derived QSAR model based on best correlated descriptors.

Drug/ Compound	Exp. LD_{50} (mg/kg)	Exp. Log LD_{50}	Pred. Log LD_{50}	Pred. LD_{50}	Connectivity Index (order 2)	Group Count (sec-amine)	Group Count (ether)
Bowellic Acid	5000	3.699	3.529	3380.65	16.184	0	0
Cichoric Acid	1750	3.243	3.379	2393.32	14.919	0	0
Levamisol	180	2.255	2.328	212.81	6.081	0	0
Lupeol			3.455	2851.02	15.556	0	0
Ursolic acid			3.533	3411.93	16.218	0	0
Oleanolic acid			3.602	3999.45	16.792	0	0

Predicted log LD_{50} (mg/kg) = +0.11890 (Connectivity Index, order 2) -0.517676 (sec. amine group count) -0.334085 (ether group count) +1.60503 [rCV^2= 0.845319 and r^2=0.97158]

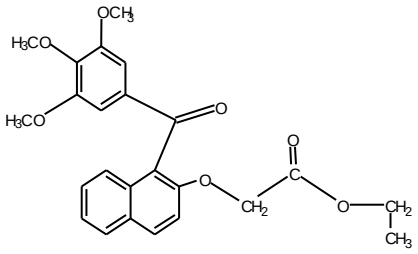
Project Title: Discovery and preclinical studies of new bioactive molecules (natural and semi-synthetic) and traditional preparations

6.8. Current status of antipsychotic herbal extract, fraction and molecules from MAP 1597 (Feroz Khan, S.K.Srivastava, M.M.Gupta)

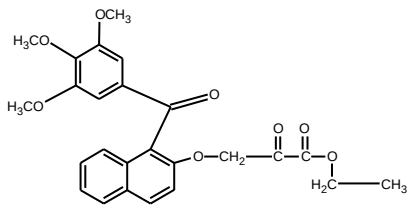
The structure of studied compounds with 79 antipsychotic drugs for drug likeness and ADMET properties similarity were compared. In drug likeness (Druggability test) we have studied fifty two physico-chemical properties such as, Rule of 5 (Lipinski's), Rule of 3, Solvation Energy, Potential energy, PSA (polar surface area) etc. In ADMET properties we have mainly studied log BB (Blood brain barrier), CNS activity (based largely on log BB), Caco-2 cell permeability etc. Using predictive QSAR models of ADMET we successfully estimated quantitative parameters of drug likeness of CIMAP molecules and derivatives.

6.9. QSAR and docking studies of gallic acid derivatives for Immunomodulatory activity (Feroz Khan, A.S. Negi)

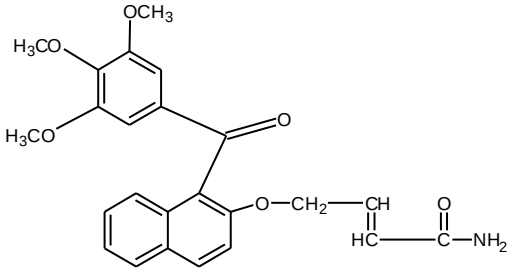
Gallic acid [3,4,5-trihydroxybenzoic acid, C6H2(OH)3COOH] exhibits a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-microbial, and anti-cancer activities. To explore the immuno-modulatory compound from derivatives of gallic acid, QSAR and molecular docking studies were performed. Theoretical results are in accordance with the *in vivo* experimental data. Relationship correlating measure of QSAR model was 99% (r^2=0.99) and predictive accuracy was 96% (rCV^2=0.96). QSAR studies indicate that dipole moment, steric energy, amide group count, lambda max UV-visible and molar refractivity correlates well with biological activity. Eight derivatives showed promising *in silico* activity and bioavailability. Docking studies also showed strong binding affinity to immuno-modulatory receptors e.g., IL-4, IL-6 and INF-α.



G-4 (Pred. LD50= 212.81 mg/kg)



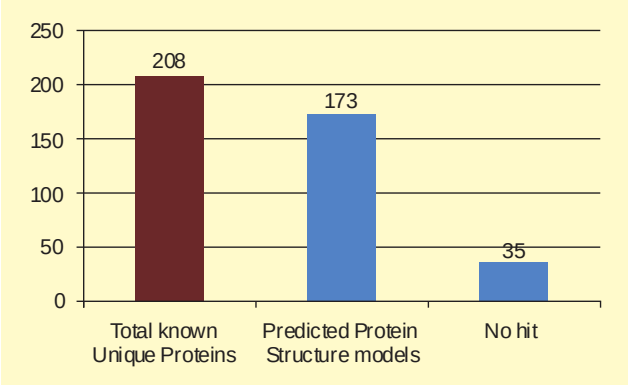
G-7 (Pred. LD50= 190.99 mg/kg)



G-9 (Pred. LD50= 325.84 mg/kg)

6.10. Malaria Project: Targets based program (Feroz Khan, Abha Meena, M.P. Darokar, A.K. Shasany, Suaib Luqman)

Total 5269 genes were scanned against human genome, out of which 4519 showed similarity with human genome and rest 750 considered unique genes. The revised status of functionally annotated unique genes are as follows: (i) functionally known genes are 208, (ii) functionally unknown genes are 473, and (iii) functionally hypothetical genes are 69. Protein structure prediction of known and functionally annotated hypothetical proteins is in progress through homology modeling and threading/fold methods, since currently only few crystal 3D structures are available in Protein DataBank.



Current status of the functionally know n proteins after 3D protein structure modeling

6.11. Structural and functional annotation of expressed sequence tags of *Papaver somniferum* (Feroz Khan, Abha Meena, Rakesh Shukla, M.P. Darokar)

In an attempt to identify additional biosynthetic steps at the molecular level, a large expressed sequence tag (EST) dataset with a total of 20,532 sequences were functionally annotated, aiming to provide sequence information for deciphering the role of genes in secondary

metabolism. Sequence cleaning filter showed 20,445 valid sequences and 87 trashed sequences at 96% minimum identity parameter for an alignment with a contaminant. Repeat masking filter showed length of valid sequences 1,69,60,151 bp, GC level 41.12% and masked bases 5,01,768 bp (2.96%) by using RepBase (update 8.12) repeats library of Arabidopsis. A total of cleaned 15,279 assembled unique transcripts were identified, which include 1,408 contigs and 13,871 singletons. Most contigs (859) were functionally annotated, while 373 contigs with hypothetical proteins and 176 contigs with unknown function were further annotated through protein family databases. We observed important genes related to secondary metabolism, which would facilitate deciphering the molecular mechanism of secondary metabolism in Papaver.

6.12. Topical anti-inflammatory activity of Agarwood oil in the TPA -Induced mouse ear inflammation (S.T. Thul, C.S. Chanotiya, Feroz Khan, D.U. Bawankule)

Topical anti-inflammatory activity of agarwood oil isolated from the *Aquilaria agallocha* was investigated using the 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced dermatitis in the mouse ear as inflammation. The oedematous response were evaluated up to 24 h after the induction and compared with the vehicle control (Acetone alone) and 2) indomethacin (200µg/ear) as representatives of non-steroid and steroid anti-inflammatory drugs, respectively. Upon GC, GC/MS and preliminary NMR analyses of the oil revealed that the bulk of the oil was constituted by agarospirol (12.5%) followed by jinkoh-eremol (11.8%) and hinesol (8.9%) as major contributor. The oil displayed strong anti-inflammatory activity against TPA -induced mouse ear inflammation model, and suggest that it may have therapeutic potential in a variety of immune-related skin diseases.

6.13. Development of target based activity screening QSAR models for alkaloids and steroidal lactones of Ashwagandha (*Withania somnifera*) (Feroz Khan, Abha Meena, N.S.Sangwan, R.S. Sangwan)

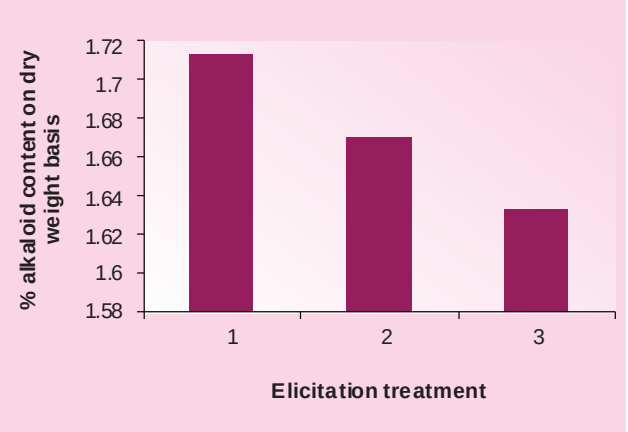
Based on reported activities, we targeted development of QSAR models for following activities such as, anti-inflammatory, anticancer, cardiac activity, antioxidant, antimicrobial, immunomodulatory, effect on central nervous system (CNS) etc. We have developed the QSAR model for human colorectal-carcinoma HCT-116 cells and (lung) cancer cell lines NCL- H460. Molecular docking studies have been done against different targets. Drug likeness and ADMET analysis are in progress.

PLANT PHYSIOLOGY

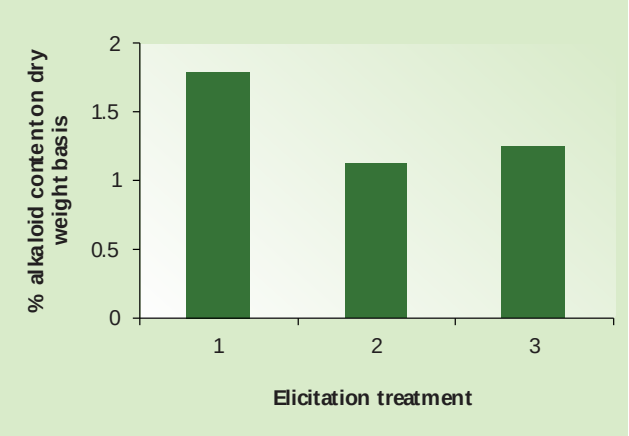
Project Title: Micronutrient–elicitor mediated metabolic modulation in *Catharanthus*.

6.14. Dose and time dependent effect of elicitation by Zn on recovery of total alkaloids from leaves and roots of *Catharanthus roseus* (N. K. Srivastava, Ajay Misra, A. K. Srivastava)

Freshly harvested leaves and roots of *Catharanthus* were exposed to different doses of micronutrient Zn at 0, 10 and 20mM for 12, 24 and 36h duration. After respective duration of treatment, leaves and roots were analyzed for total alkaloid content.



Dose and time (24h) dependent effect of Zn elicitation on recovery of total alkaloids from leaves. Legends- 1- 0mM Zn, 2-10mM Zn, 3-20mM Zn. LSD values at 5% level 0.264.



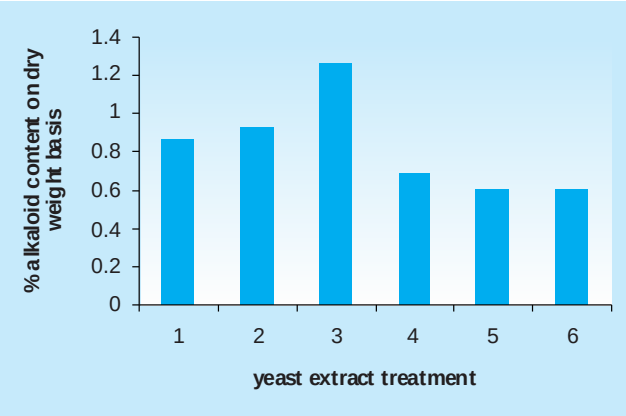
Dose and time (24h) dependent effect of Zn elicitation on recovery of total alkaloids from roots. Legend- 1- 0mM Zn, 2- 10mM Zn, 3-20mM Zn. LSD values at 5% level 0.581.

Project Title: Post harvest studies during leaf processing on the recovery of total alkaloids in *Catharanthus roseus*.

6.15. Effect of yeast extract on alkaloid recovery in post harvested leaves of *Catharanthus* (N. K. Srivastava, Ajay Misra, A. K. Srivastava)

Harvested leaves of *Catharanthus* were treated with

different doses of yeast extract (YE) to determine optimum dose leading to maximal recovery of total alkaloids. The doses applied were, 1- control (0g/l YE), 2- 3g/lYE, 3- 5g/lYE, 4- 7g/lYE, 5-9g/l YE, 6-11g/lYE. After treatment, the leaves were dried and the total alkaloids were determined.



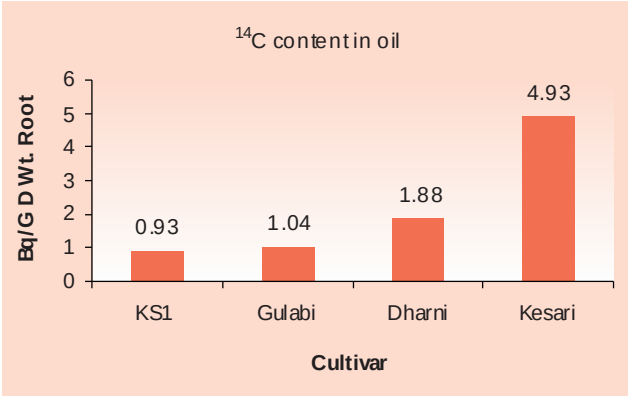
Dose dependent effect on the recovery of total alkaloids from post harvested leaves. Legends- 1- control (0g/l YE), 2- 3g/lYE, 3- 5g/lYE, 4- 7g/lYE, 5-9g/l YE, 6-11g/lYE. LSD values at 5% 0.260.

This was also optimized for time (0, 12, 24, 36, 48, 60h of 5g/lYE treatment) and maximum increase in alkaloids was observed in 24h treatment.

Project Title: Photosynthetic metabolism in cultivars of Vetiver.

6.16. Variations among commercial cultivars of vetiver (*Vetiveria zizanoides* L) in the photosynthetic and metabolic characters associated with essential oil accumulation (N. K. Srivastava, Ajay Misra, A. K. Srivastava)

Intraspecific variations in four widely cultivated cultivars of vetiver (KS1, Kesari, Gulabi and Dharni) (*Vetiveria zizanoides* L) were analyzed to understand the physiological basis of regulation of essential oil accumulation. Feeding studies revealed that in cultivar Kesari ^{14}C content in oil was higher (4.93 Bq/g root d wt) than in cultivar KS1 (0.93 Bq/g root d wt). The leaf photosynthetic rate showed significant positive association with root biomass (0.766). The ^{14}C label in metabolite sugar showed significant positive association with oil content in root (0.971), root biomass (0.739) and oil yield (0.919). Efficient cultivar translocated a higher portion of leaf carbon assimilate towards the root and towards the biosynthesis of essential oil.



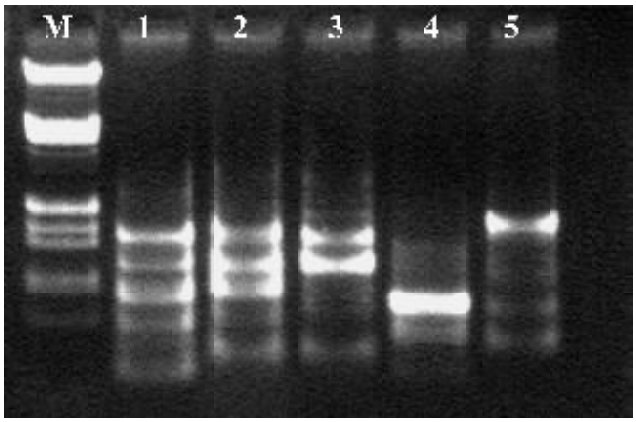
Incorporation of ^{14}C label into root essential oil in different cultivars of Vetiver. SEM-1.58 and SED-2.23.

TAXONOMY & PHARMACOGNOSY

6.17. Identification and characterization of natural hybrid(s) of *Sida* species occurring in North Indian plains (G.D. Bagchi, Abdul Samad)

A new species / variety of *Sida* identified on the basis of phenotypic, reproductive, molecular and chemical characters. The new population phenotypically resembled with *S. acuta*. RAPD analysis also showed higher genetic similarity of this population with *S. acuta* (similarity coefficient 0.678) than *S. alba* (0.521). Relatively lower genetic similarity of this population has been noted with *S. cordifolia* and *S. cordata* (0.489 and 0.478 respectively). The four aforementioned species demonstrated normal flowering and up to 98-99% seed formation, while the plants of the new populations exhibited self incompatibility due to twirling and upward movement of the pollen tubes at the upper region of the ovary. Such abnormality led to extremely low seed

New *Sida* species / variety



RAPD profile of different *Sida* species
(M- λ EcoR1 *Hind*III double digest marker,
1- *S.acuta*, 2- *Sida* new population, 3- *S.alba*,
4- *S.cordata*, 5- *S.cordifolia*

formation (1-2%). Chemical profiling further exemplified the distinctiveness of this population as it expresses the hybrid-like chemical profile between *S. acuta* and *S. alba.*

Project Title: Conservation to bio-prospecting genes / molecules / products

6.18. Domestication and evaluation of two rare varieties of *Artemisia roxburghiana* at sub-tropical conditions of north Indian plains (G.D. Bagchi)

Two varieties of *A. roxburghiana* i.e. var. *hypoleuca* and var. *purpurascens* have been collected from Himachal Pradesh (1982m) and Uttarakhand (2000m) respectively. Plants of both the varieties were domesticated at the sub-tropical condition of Lucknow and their oils were analyzed.

Both varieties exhibited extreme changes in their oil constituents at sub-tropical conditions from the oils of natural plants. At natural habitats, the major oil constituent of var. *hypoleuca* were β-thujone (65.3%). While, at sub-tropical condition, major oil constituent became α-humulene (20.0 %). Increase in the α-humulene content at sub-tropical condition, increased the odour value of the oil.

A- *Artemisia roxburghiana* var. *hypoleuca*
B- *Artemisia roxburghiana* var. *purpurascens*

Oils of naturally growing plants of var. *purpurascens* growing at higher altitude were rich in borneol (21.2%) and linalyl acetate (7.4%). Plants growing at the lower altitude contained β-caryophyllene (18.0%) and α-thujone (12.0%). Plants when domesticated at sub-tropical conditions became rich in camphor (48.3%), which enhanced its commercial odor value.

7. RESEARCH CENTRE, BANGALORE

7.1. Quantitative Assessment of Carbon Sequestration by different Aromatic Crops under field conditions and controlled conditions representative of climate change (Munnu Singh, E.V.S. Prakasa Rao, R.V. Sreedhar)

A field experiment was conducted during 2010-11 at CIMAP Research Centre, Bangalore to study carbon sequestration by Lemongrass (*Cymbopogon flexuous*). Destructive plant samples were taken once in 60 days and at harvest. Dry matter and organic carbon was estimated in shoot and root biomass. Total organic carbon 5.38 t/ha was sequestered by Lemongrass.

Organic Carbon sequestration in Lemongrass

Particulars	Organic carbon (%)	Dry Matter (t/ha)	Carbon Sequestered (t/ha)
Lemongrass (shoot)	46	10.5	4.83
Lemongrass (root)	35.5	1.57	0.55
Total (carbon sequestered)	**-**	**-**	**5.38**

7.2. Genotype designing for specialty/opportunity crops in medicinal and aromatic plants (Ramesh Kumar)

Linalool rich genotype (76.395 % linalool content) of *Ocimum basilicum* having citral note due to presence of high citral (total citral content – 11.291%) was subjected to selection for high linalool and less/ nil citral variants. Three supra-linalool variants; T-4, T-5 and T-15 with linalool contents of 92.150%, 87.573% and 89.304% respectively were identified. The citral contents of the selects were relatively lower than the parental population; T-4 (total citral - 0.023%), T-5 (total citral - 0.145%) and T-15 (total citral - 0.277%). The promising variants maintained in isolation will be subjected to further selections and yield trails for development of supra-linalool cultivar. The oil composition is presented given below:

Linalool and citral contents of parental population and promising selections in Ocimum *basilicum*

SN	Parent/ Selections	Linalool Content (%)	Citral Content (%)		
			Citral-I	Citral-II	Total Citral
1	Parental Population	**76.395**	4.961	6.330	**11.291**
2	T-4	**92.150**	0.018	0.005	**0.023**
3	T-5	**87.573**	-	0.145	**0.145**
4	T-15	**89.304**	-	0.277	**0.277**

7.3. Gene bank utilization strategies: Conservation to bio-prospecting genes/molecules/products (Ramesh Kumar)

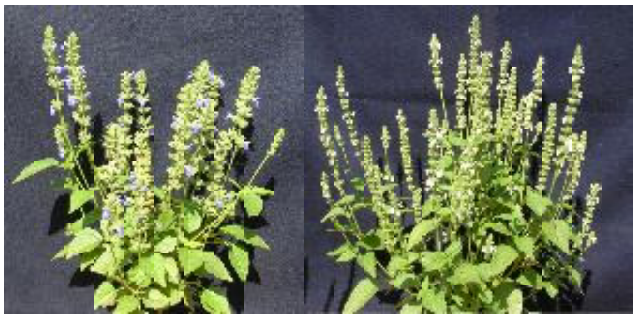
Breeding work on Ashwagandha (*Withania somnifera*)

was initiated at CRC, Bangalore with an objective to develop cultivars with high root yield having good physical texture. In this regard germplasm lines obtained from CIMAP, Lucknow; promising selections from previous year trials; varieties and cultivated accessions were maintained and characterized.

Enormous variation for morphological features such as berry colour, plant height, leaf morphology, stem branching, capsule morphology, etc. were observed. Morphotypes with distinct morphological features were identified and characterized. The morphotypes with contrasting morphological features will be subjected to evaluation and genetic studies.

7.4. Genetic improvement of Chia (*Salvia hispanica* L.) and *in vitro* evaluation of the biosynthetic pathway of Omega 3 fatty acids (Sreedhar R.V. and Malathi Srinivasan)

As an effort for genetic improvement of chia, assessment of genetic variability has been undertaken. Variation in major yield attributing characters such as number of inflorescence per plant, number of flowers per inflorescence, inflorescence length and number of branches per plant has been observed. For isolation of pure lines, hundred individual plants have been selected based on their superior phenotypic characters. The white and blue flowering types of chia have been isolated and are under field trials for evaluation of their qualitative and quantitative characters. Induction of genetic variability through mutation has also been attempted and the LD_{50} value for gamma irradiation has been standardized for chia. The mutant lines obtained are under field evaluation.



The blue & white flowering types of Chia (*Salvia hispanica* L.)

Variation in inflorescence length (a) and number of flowers per inflorescence (b) in Chia (*Salvia hispanica* L.)

7.5. Genetic studies for improvement of periwinkle, Catharanthus

7.5.1. Genetic relationship between morphological marker traits of two independently induced mutants with high content of alkaloid in leaves and roots (R.N. Kulkarni, K. Baskaran)

To study relationship between genes for high content of alkaloids in two independently induced mutants, 'necrotic leaf mutant' and mutant No. 7 and to study the possibility of combining them into a single genotype, the mutants were hybridized. F_2 seeds were produced through manual selfing of F_1 plants. F_2 generation was raised and scored for morphological mutant marker traits, *viz*., 'necrotic leaf lesions' of 'necrotic leaf mutant' and 'nerium' leaf lamina of mutant No.7. These mutant marker traits were found to be governed by independently inherited monogenic recessive genes.

7.5.2. Relationship between genes increasing and reducing plant height and determination of their individual and combined effects (R.N. Kulkarni, K. Baskaran)

The genetic relationship between an extremely tall mutant (EMS 18-12) and a bushy mutant (EMS 24-5) was studied. Two independently inherited epistatic genes were found to govern extreme tall phenotype in the mutant EMS 18-12 and were found to be inherited independently of the recessive gene governing bushy phenotype in the mutant EMS 24-5.

The two mutants, their parental variety, Nirmal and the double mutant recombinant were evaluated to study the effect of the genes involved in plant height. It was found that the genes involved in the extremely tall mutant increased plant height about 90% (from 74 to140 cm) while gene in the bushy mutant reduced plant height by about 25% (from 74 to 56 cm), as compared to the parental variety, Nirmal (74 cm). The height of the double mutant recombinant (129cm) was greater than the mid-parental value (98cm) and the height of the parental variety, Nirmal (74cm) suggesting that the effect of height increasing genes was greater than the effect of the height reducing gene. The contents of alkaloids in both leaves (1.06%) and roots (1.90%) of the double mutant recombinant were similar to those in the parental mutants (1.18 and 1.17% in leaves and 1.96 and 1.93% in roots of extremely tall and bushy mutant, respectively) as well as the parental variety, Nirmal (1.07 and 2.00% in leaves and roots, respectively).

7.6. Genetic enhancement of patchouli

7.6.1. Multiplication of patchouli clones exhibiting greater tolerance to mannitol induced stress *in vitro* than the parental variety (R.N. Kulkarni)

Two somaclones PMI 54 & PMI 57 regenerated on MS medium supplemented with 60 mM mannitol *in vitro* had exhibited greater tolerance to mannitol induced stress than the parental variety when evaluated *in vitro*. These clones were taken up for *in vitro* multiplication. Altogether about 100 plants of these two and another somaclone PMI 118 (regenerated initially on 60mM mannitol medium and subsequently subcultured on 20mM mannitol medium) have been produced for further multiplication for taking up evaluation of their tolerance to moisture stress *in vivo*.

8. RESEARCH CENTRE, HYDERABAD

8.1. Intercropping lemongrass (*Cymbopogon flexuosus*) with vegetables for higher income generation (B.R. Rajeswara Rao, D.K. Rajput)

A field experiment was conducted with lemongrass cv. Cauvery intercropped with cluster bean, coriander, lady's finger, green amaranth, fenugreek, sorrel leaf and spinach during 2008-2010 for higher income generation under the semi-arid tropical climate. Total biomass and essential oil yields of lemongrass were not adversely affected by intercropping.

8.2. Response of *Phyllanthus amarus* cv. CIM Jeevan to nitrogen application (B.R. Rajeswara Rao, D.K. Rajput)

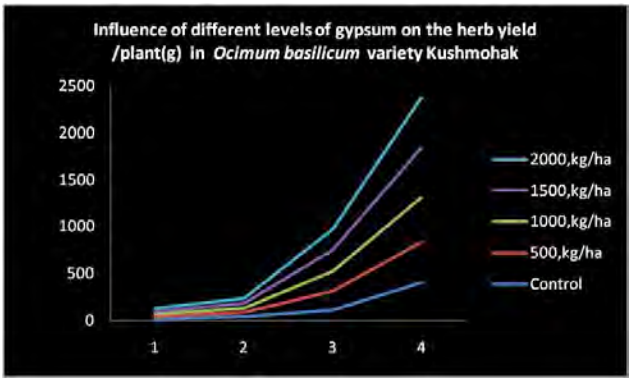
The results of the first year field experiment carried out during the rainy season of 2010-11 with cultivar CIM-Jeevan revealed that nitrogen application (30-120 kg/ ha) significantly increased yield attributes and biomass yield of *Phyllanthus amarus* in comparison to control.

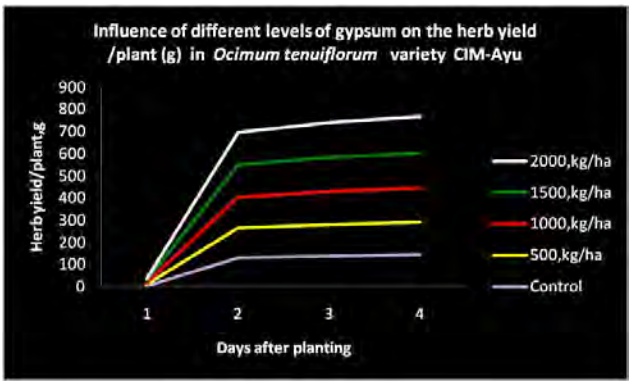
8.3. Mealy bug infestation on prickly pear (*Opuntia dillenii*) (B.R. Rajeswara Rao, D.K. Rajput)

Prickly pear is an edible, dye yielding, medicinal plant that grows in dry areas. During the current year majority of the collected plants were severely affected by mealy bugs resulting in their death. Samples were given to the entomology department of Acharya N.G. Ranga Agricultural University, Hyderabad for the identification of the species. This is the first report of mealy bug infestation on prickly pear.

8.4. Influence of different doses and methods of application of gypsum on the growth and essential oil yield of *Ocimum basilicum* variety Kushmohak and *Ocimum tenuiflorum* variety CIM-Ayu (K.P. Sastry)

Two experiments were conducted to study the influence of different levels and method of application of gypsum on soil remediation and the growth and essential oil yield of *Ocimum basilicum* variety Kushmohak. Application of 1500 kg of gypsum/ ha resulted in significant improvement in the herb yield. Spot application of gypsum in concentrated spots resulted in higher herb yield compared to the other methods *viz*., broadcasting, line placement or incorporation.





8.5. Influence of different levels of vermicompost on root initiation and growth of rose scented geranium cuttings (K.P. Sastry)

Two experiments were conducted to study the influence of different doses of vermicompost (0-800 kg/ha) on the root initiation and growth of terminal stem cuttings in rose scented geranium nursery for a period of ninety days. Vermicompost (@ 800 kg/ha) application resulted in faster induction of roots compared to control. It also significantly influenced the number (1.33-3.00) and length of roots (0.16- 0.72 cm) in geranium. Number of leaves/ cutting, weight of cutting and weight of stem were also significantly and positively influenced by the treatment.

8.6. Influence of different concentrations of growth hormones on the root initiation and growth of *Bacopa monnieri* (K.P. Sastry)

A series of experiments were conducted to study the effect of different concentrations of IAA (0-200 ppm) on root initiation and growth in Bacopa. In *Bacopa monnieri* root initiation and growth were influenced significantly due to treating the cuttings with IAA 200 ppm for 20 minutes before planting. The root initiation was faster and the number of roots produced was significantly more due to IAA 200 ppm compared to all other treatments.

8.7. Influence of different concentrations of IBA on the root initiation and growth of long pepper (K.P. Sastry)

In an experiment conducted to study the influence of five concentrations of IBA (0, 50, 100, 150 and 200 ppm) on root initiation and growth of cuttings in the nursery, root initiation started at 11 days after planting and continued up to 38 days after planting. Increasing the concentration of IBA from 0 to 200 ppm increased the number of roots/cutting. Highest number of roots (6.50/ cutting) were produced at 200 ppm concentration at 38 days after planting. The average length of root/ cutting also increased from 0.38 cm in case of control to 1.66 cm in case of IBA 200 ppm treatment. IBA treatment has induced formation of leaves also besides roots from 11 days after planting.

8.8. Strategic rural income enhancement by medicinal and industrial plants based technologies (K.P. Sastry)

Medicinal and Aromatic plants are promoted extensively in the rural areas of Andhra Pradesh. Director, CIMAP distributed Aswagandha seeds to the farmers in an interactive meet on 16th June, 2010. Three awareness camps, one training programme and one market exposure visit were conducted during 2010. Vetiver planting material sufficient for 10 acres was distributed to thirty tribal farmers in the East Godavari district of Andhra Pradesh.

8.9. Chemical investigation on *Plectranthus bishopianus* Gamble, a rare and endemic plant and bio activity studies (K.V. Syamasundar, M.P. Darokar, Alok Kalra)

The chemical investigation of *Plectranthus bishopianus* with systematic bio guided fractionation studies was undertaken to isolate the bioactive compounds. Three diterpenoids, two sterols and one triterpene were identified by spectral studies. 6β-hydroxy – 7α-methoxyroyleanone is a new compound. The other compounds identified are 6β, 7α-dihydroxyroyleanene, 6β, 7α–dihydroxyroyleanene, β-sitosterol, stegmasterol and oleanolic acid and it is first time reported from this plant.

8.10. Processing of medicinal and aromatic plants for isolation of lead drug molecules at pilot scale level - Bio guided fractionation and chemical investigation of *Hyptis suaveolens* L Poit for bio-active molecules (K.V. Syamasundar, B. Balakishan)

The hexane, chloroform and ethyl acetate extracts showed good antioxidant activities. Column chromatographies of the hexane extract afforded two sterol derivatives and were identified as β-sitosterol and stegmasterol by NMR and MS studies.

8.12. Phytochemical investigation of Bioactive compounds from medicinal plants of Deccan plateau region (J. Kotesh Kumar, K.V.N.S. Srinivas, A. Niranjan)

Phytochemical investigation of some of the common, rare and endangered medicinal plants of Deccan plateau region led to the isolation of pure compounds whose structure elucidation and bioactivity studies are in progress. The plants include: *Sizygium alternifolium*, *Euphorbia heterophylla*, *Tridax procumbens*, *Euphorbia fusiformis*, *Silla sps*, *Syzygium mundagum*, *Annona squamosa*.

9. RESEARCH CENTERS, PANTNAGAR AND PURARA

9.1. Cultivation Potential of three Rose-scented Geranium (*Pelargonium graveolens*) cultivars in the Kumaon Region of Western Himalayas (Amit Chauhan, R.S. Verma)

A field experiment was conducted to evaluate the production potential of three cultivars of rose-scented geranium *viz*., 'Bourbon', 'CIM-Pawan' and 'Kelkar' in the temperate region of Uttarakhand. 'CIM-Pawan' had the highest essential oil yield (103.87 g plot^{-1}) followed by 'Kelkar' (79.93 g plot^{-1}) and 'Bourbon' (72.01 g plot^{-1}). The essential oil profile of 'Bourbon' was rich (relative percentages) in citronellol (29.05), geraniol (24.36), citronellyl formate (5.94), isomenthone (5.82); the oil of 'CIM-Pawan' was rich in citronellol (32. 60), geraniol (21.38), 10-epi-α-eudesmol (6.83), citronellyl formate (6.29) while the essential oil of 'Kelkar' showed a different profile with citronellol (61.48) and isomenthone (10.56) being almost twice that of other cultivars.

9.2. Chemo profiling and quality evaluation studies on commercial elite cultivars of aromatic plants (R.C. Padalia, R.S. Verma, Amit Chauhan, V. Sundaresan)

9.2.1. Assessment of variability in volatile constituents of peppermint cultivars and some wild accession from northern India

The terpenoid composition of elite cultivars of peppermint (*Mentha piperita* L.) grown in foot and mid hill conditions of Uttarakhand (India), and accessions collected from wild were analyzed and compared. The major characteristic constituents of all the cultivars and wild accessions were menthol (30.3-47.8%), menthone (4.5-48.6%), menthyl acetate (1.0-9.5%), menthofuran (0.1-14.6%), 1,8-cineole (4.1-8.9%), neo-menthol (1.5-4.9%) and *iso*-menthone (1.2-3.9%). Quality evaluation of peppermint oils revealed that the performances of all cultivars grown at foothill conditions are very close to the requirements of European pharmacopoeia as compared to mid-hills.

9.2.2. Chemical fingerprinting of the fragrance volatiles of nineteen Indian cultivars of *Cymbopogon* Spreng. (Poaceae)

Essential oil content and composition of 19 commercial cultivars of *Cymbopogon* Spreng. (Poaceae) species *viz*. *C. martinii*, *C. flexuosus*, *C. winterinus*, *C. pendulus* and a hybrid of *C. khasianus* x *C. pendulus* were examined and compared using capillary GC-FID and GC-MS. The comparative results showed considerable variation in the qualitative and quantitative compositions of essential oils. On the basis of chemical similarity the cultivars of genus *Cymbopogon* was divided into five chemical variants/groups within two series *viz*. *Citrati* and *Rusae*.

9.2.3. Study on ontogenic variation in volatile constituents of *Artemisia annua*

An experiment was conducted to observe the variations in essential oil yield and composition of the aerial parts of *A. annua* var. CIM-Arogya grown in tarai conditions of Uttarakhand, India at different stages of growth. The essential oil content was found to vary from 0.35% to 0.75% at different crop stages. Essential oil analysis showed monoterpenoids (38.5%-72.0%) and sesquiterpenoids (22.2%-48.2%) as major constituents. The major constituents identified were camphor (22.8%-42.6%), 1,8-cineole (3.7%-8.4%), linalool (<0.1%-11.9%), β-caryophyllene (2.0%-9.2%), (*E*)-α-farnesene (1.3%-8.5%), germacrene D (0.5%-7.3%) and 1-*epi*-cubenol (0.7%-5.2%) at different crop stages. The results of yield and quality of essential oils of *A. annua* var. CIM-Arogya was shown to be strongly dependent on crop stages, with flowering and seed setting stages as the best harvesting time for the maximum content and quality essential oils.

9.3. Chemical and biological activity evaluation of aromatic spices of Uttarakhand (R.C. Padalia, R.S. Verma, Amit Chauhan, V. Sundaresan)

9.3.1.Chemical constituents and antibacterial and antioxidant potential of *Zingiber officinale*

The essential oil composition of the leaves and rhizomes of *Z. officinale* were analyzed and compared by capillary GC-FID and GC-MS. β-Caryophyllene (22.65%), geranial (11.26%), caryophyllene oxide (9.51%), geraniol (8.29%) and neral (7.44%) were the major constituents identified in leaf oil; while zingiberene (15.92%), geranial (11.75%), α–neral (9.25%), β-phellandrene (7.73%), β-bisabolene (11.12%) and β-sesquiphellandrene (5.26%) in rhizome oils of *Z. officinale*. The essential oils showed moderate antibacterial activity against four bacterial strains *viz*. *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis, Klebsiella pneumoniae* and *Bacillus subtilis*. The antioxidant activity screening of rhizome oil of

Z. officinale was also tested by DPPH radical scavenging system.

9.3.2. Comparative essential oil composition and antibacterial activity of leaf and seed oils of *Coriandrum sativum*

The leaf and seed essential oils of *Coriandrum sativum* L. (family: Apiaceae) were analyzed; and 89 constituents identified forming 93.55% to 98.90% of oil compositions. The major components in seed essential oil were monoterpenoids (90.07%) *viz*. Linalool (55.42%), geranial (6.33%), myrcene (5.53%), α-humulene (5.29%) and 1,8-cineole (3.30%). On the contrary, aliphatic compounds (90.20%), mainly comprised of C_8-C_{16} aldehydes and alcohols, predominate in leaf essential oil of *C. sativum*. The major constituents identified were *(E)*-2-decenal (18.02%), decanal (14.36%), dec-9-en-1-ol (11.66%), (*E*)-2-dodecenal (8.72%), (*E*)-2-tridecenal (6.09%), dodecanal (5.81%) and decanol (5.77%). Comparison of the oil composition of seed and leaf oil showed sharp qualitative and quantitative variation in their essential oil constituents. The bioassay showed that the seed essential oil showed significant activity against *Escherichia coli*, *Enterococcus faecalis, Klebsiella pneumoniae,* and *Bacillus subtilis*

9.4. Phytochemical investigation of the lesser known Aromatic plants of India (R.S.Verma, R.C. Padalia and Amit Chauhan)

9.4.1. Chemical investigation of the essential oil of *Hypericum japonicum*

Hydrodistilled essential oil of the aerial parts of *Hypericum japonicum* Thunb. ex Murray, grown in northern India was analyzed by GC and GC-MS. A total of seventy constituents representing 93.6% of the total composition have been identified. Major constituents of the oil were 2-methyl octane (24.9%), *n*-nonane (21.4%), and (2*Z*)-nonenol (16.5%).

9.4.2. Chemical investigation of *Laggera crispata* essential oil

Hydrodistilled essential oil of the aerial parts of *Laggera crispata* (Vahl) Hepper & Wood, collected from Uttarakhand was analyzed by GC and GC-MS. Eighty constituents, accounting for 83.9% of the oil composition were identified. The oil was mainly dominated by sesquiterpenoids (45.3%) and benzenoid compounds (33.9%). Among them, 2,5-dimethoxy-*p*-cymene (32.2%), 10-*epi*-ν-eudesmol (14.7%), and β-caryophyllene (6.9%), were major components.

9.4.3. Chemical composition and antimicrobial activity of the *Capillipedium parviflorum* inflorescence essential oil

Essential oil isolated from the inflorescence of *Capillipedium parviflorum* (R. Br.) Stapf, growing wild in Uttarakhand, was investigated by GC and GC-MS. A total of forty five constituents representing 99.0% of the oil were identified. The major components of this oil were 4-undecanone (33.2%), 4-undecanol (29.7%), 4-nonanol (13.9%), and α-murolol (5.3%). The bioassay showed that the essential oil possesses good antibacterial activity.

9.4.4. Chemical fingerprinting of the leaf, flower and stem essential oils of *Salvia officinalis*

Volatile oils of leaf, flower and stem of the *Salvia officinalis* L., grown in Uttarakhand were analyzed by GC and GC-MS. A total of sixty-two constituents representing 95.55-97.37% of the total oil compositions were identified. Major components of the oils were (*Z*)-thujone (19.78-42.46%), (*E*)-caryophyllene (4.93-16.14%), manool (5.41-15.08%), viridiflorol (6.74-12.75%), 1,8-cineole (2.77-12.88%), and camphor (1.35-11.31%). Comparative results showed considerable variation in the essential oil composition of leaf, flower and stem.

9.4.5. Essential oil profile of *Stevia rebaudiana* (Bert.) Bertoni roots

The essential oil isolated from roots of *S. rebaudiana* was analyzed by GC and GC-MS. A total of sixty components, representing 86.57% of the total oil composition were identified. Major components of this oil were α-longipinene (22.58%), α-isocomene (15.36%), modheph-2-ene (14.41%), (*Z*)-caryophyllene (6.18%), silphinene (6.08%), and α-amorphene (2.91%).

9.4.6. Chemical investigation of *Thymus linearis* essential oil

Thymus linearis (Benth. ex Benth) were collected from five distinct locations of Uttarakhand during summer season. The hydro-distilled essential oil (yield 0.84-0.95%) was analyzed by GC and GC-MS. A total of 56 constituents representing 81.55-98.11% of the total oil composition were identified. Thymol (52.28-66.65%), *p*-cymene (1.81-21.60%) and ν-terpinene (1.94-12.48%) were the major constituents in all populations.

9.5. Chemical characterization and biological activity evaluation of decanted and hydrophilic fractions of essential oils (R.S.Verma, R.C. Padalia and Amit Chauhan)

9.5.1. Chemical profile and antimicrobial potential of hydrophilic volatiles of *Thymus serpyllum* and *Thymus linearis*

Thymus serpyllum L. and *Thymus linearis* Benth. ex Benth., cultivated in Uttarakhand was hydro-steam distilled in small size field distillation unit. The secondary volatile oils of *T. serpyllum* and *T. linearis* were richer in phenolic monoterpenes (92.3% and 96.4%, respectively) as compared to primary volatile oils (42.1% and 44.9%, respectively). The bioassay showed that the secondary oils of both the *Thymus* spp exhibited stronger antibacterial and antifungal activities than primary oils.

9.5.2. Chemical composition and antimicrobial potential of aqueous distillate volatiles of Indian *Mentha piperita* and *Mentha spicata*

The decanted (main essential oil) and hydrophilic (recovered essential oil) fractions of *Mentha piperita* L. *cv Kukrail* and *Mentha spicata* L. *cv MSS-5* essential oils were investigated by GC and GC-MS. In *M. piperita*, oxygenated monoterpenes, especially alcohols were observed to be higher in recovered oil than decanted oil. Similarly, in *M. spicata* the percentage of oxygenated monoterpenes was quite higher in recovered oil (83.08%) than decanted oil (59.84%). The recovered and decanted essential oils of both *Mentha* spp demonstrated moderate to good antimicrobial activity.

10. TECHNOLOGY & BUSINESS DEVELOPMENT

HERBAL MEDICINAL PRODUCTS

Project Title: Development of standardized herbal formulations for better health

10.1. Evaluation of turmeric and onion extracts against TPA (*o*-tetradeconylphorbol 13-acetate) induced inflammation in small animals (N. P. Yadav)

The present investigations provide evidence that the alcoholic extract of *Curcuma longa,* dichloromethane (DCM) extract of *Allium cepa* and mixture of extract of both plants are topically active in attenuation of inflammation induced by TPA. Extracts of plants resulted in marked inhibition of eight important events related to the skin inflammatory response induced by TPA namely ear redness, ear edema, molecular formation of MDA, accumulation of NO, and an increase in tissue level of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 & IFN-γ), in a manner similar to Indomethacin, the reference anti-inflammatory drug. Extracts also significantly increased the levels of anti-inflammatory cytokine i.e. IL-10.

10.2. Formulation for household based treatment of portable water for domestic use (A.K. Singh, D. Mani, N.P. Yadav)

Moringa oleifera seeds are non-toxic, and recommended for use as coagulant in water treatment which can produce efficient reduction of turbidity (80.0% to 99.5%) with 90.00% to 99.99% bacterial reduction.

In rural and areas water used for domestic purposes is highly contaminated and unsafe. Through scientific validation some potent synergistic combination of natural products along with *Moringa oleifera* has been developed which is capable of clarifying of turbid water in an easy-to-use sachet.

Project Title: Bio-prospection of phyto molecules, extracts

10.3. Evaluation of antioxidant and free radical – scavenging activities (A.K. Singh, Suaib Luqman)

In order to identify new sources of safe and inexpensive antioxidants in herbal formulation, the antioxidant capacity of six different extracts each from the plants *Callicarpa macrophylla* and *Rabdosia melissoides* were evaluated. The inhibition of induction ODC (epidermal ornithine decarboxilage) activity was maximum in non polar fractions, AAE (ascorbic acid equivalent activity) and GAE (gallic acid equivalent activity) was maximum in ethyl acetate extract. However maximum Free radical –scavenging activity (DPPH) was recorded in alcohol extract. The studies reveal that besides extracts, essential oils of *Rabdosia melissoides* can be potent candidate for antioxidant activities.

10.4. Evaluation of calliterpenone on medicinal and aromatic crops (A.K. Singh, G.D. Bagchi, Amitabh Chattopadhyay)

Effect of calliterpenone on *Andrographis paniculata* and *Ocimum basilicum* was carried out in pot and field experiments. Besides enhancement in root, shoot and total biomass, 0.001mM solution of calliterpenone was found to be effective in enhancing the total chlorophyll and carotenoid content in leaves of *Andrographis paniculata* by 57.84% and 67.9% respectively. However other growth promoters NAA and IAA also enhanced chlorophyll content in leaves by 66.9% & 63.7% and carotenoid content by 56.3% & 50.4%. But the important point was that calliterpenone produced these results at ten times lower dilution as compared to others. However, the total andrographolide content was not affected by calliterpenone.

In *Ocimum basilicum*, the chlorophyll and cartenoid contents were enhanced by 0.001 mM solution of calliterpenone application, compared to no effect by other growth promoters.

TECHNOLOGY TRANSFER

(A.K. Singh, V.K.S. Tomar, Alok Krishna, R.P. Bansal, Sanjay Kumar, Ram Suresh, Dinesh Kumar, R.P. Yadav)

CSIR-CIMAP Kisan Mela – 2011

CIMAP - Kisan Mela organized on 31st January, 2011 was attended by about 1500 people from different strata of society including farmers and entrepreneurs hailing from different states such as Uttar Pradesh, Bihar, Haryana, Punjab, Rajasthan among others. Approximately 500 farmers got themselves registered for CIMAP agro- advisory SMS service launched on the occasion. On this occasion improved variety of *Stevia* – 'CIMAP Madhu' was released. The main attraction of

the Kisan Mela was the participation of the farmers and entrepreneurs who displayed their products specially agarbattis and aloe gel made by them. A compendium of improved agrotechnology of commercially viable medicinal and aromatic plants, 'Aus-Gyanya' was also released on this occasion.

Consultancy and technical services rendered:

SN	Name of the party	Detail of the consultancy / technical service	Amount received (Rs. in Lakh)
1	West Bengal Pharmaceutical and Phytochemical Development Corporation Limited (WBPPDCL), Kolkata	Survey of the site of the company in North Bengal and preparation of feasibility report	3.30
2	IPCA Laboratories Limited, Ratlam	Contractual cultivation of *Artemisia annua* plant in Uttar Pradesh and Uttarakhand	7.03 *
3	Chiara Herbals Private Limited, New Delhi	Technical guidance for preparation of liquid dentifrice	6.00
4	U. P. Export Promotion Bureau, Lucknow	Organisation of Mint Meet in district Badaun of Uttar Pradesh	1. 63
5	Saf Yeast Co. Pvt. Ltd., Sandila, Hardoi	Preliminary survey and technical advice for cultivation of aromatic plants in their factory premises	0.25

* In addition to consultancy charges, Rs. 7.50 lakh received for *A. annua* seeds of variety 'CIMAP – Arogya' for sowing on the farmers' field selected by the company.

Participation in exhibitions/fairs/expositions

SN	Title	Dates	Location
1.	CSIR Technofest 2010	14-28 November, 2010	India International Trade Fair, Pragati Maidan, New Delhi
2.	ASSOCHAM Herbal - Expo	14-16 January, 2011	NSIC Exhibition Complex, Okhla, New Delhi
3	CIMAP - Kisan Mela 2011	31 January, 2011	CIMAP, Lucknow
4.	Nutraceutical Summit and Expo	15-18 February, 2011	World Trade Centre, Mumbai
6.	State Flower and Vegetable Show	19-20 Febuary, 2011	Governor House, Lucknow

Training programs on cultivation and processing of economically viable medicinal and aromatic plants

SN	Dates	No. of partici-pants	Sponsoring Agency
1.	11-12 January 2011	30	UPDASP
2.	06-07 January 2011	25	U-SERC
3.	17-21 January 2011	30	ATMA
4.	29-31 January 2011	24	HMMP
5.	27-28 December 2010	19	U-SERC
6.	9-10 December 2010	25	Uttar Pradesh Rajya Mahila Ayog
7.	10-12 November 2010	6	CIMAP
8.	28-30 October 2010	52	SIDBI
9.	28-30 September 2010	31	SIDBI
10.	14-15 September 2010	13	CIMAP
11.	17-18 June 2010	18	U-SERC
12.	14-16 June 2010	26	NHM
13.	26-27 May 2010	21	U-SERC

Survey and technology interventions

Niwari, District Tikamgarh, Madhya Pradesh, 27 November 2010

Conducted survey of Kisni, Prathvipur, Niwari, Devra, Poha, Babai, Uprara, Kanchanpura, Garkundar, Madori, Majal, Ajadpura, Mudara, Churari, Kalua, Teharka etc. and interacted with more than 100 farmers. Most of the farmers were growing rain fed wheat, mustard, Urd, Mung, Makka and vegetables. Farmers are dependent on rainwater and ponds. Farmers were told to introduce MAPs crops under rain fed condition. Production and processing technology of Some important MAP suitable for Bundelkhand region delivered by Dr VKS Tomar and Dr Alok Krishna. The main crops Lemongrass, Palmarosa, Basil, Kalmegh, Satavar, Ashwagandha, Sarpagadha were discussed in details.

Moth, Bangra, Jhansi U.P and Bhabua, Aita, district Jalaon, 16 December 2010

In order to provide benefit of integration of MAPs in existing cropping system, CIMAP interacted with the farmers of Moth, Barusagar, Bangra district Jhansi, and Bhabua, aita, district Jalaon, U.P. Minterop is being cultivated in Jalaun. It is observed that water table is depleting due to frequent irrigation. Hence CIMAP is discouraging the farmers for mint cultivation. The farmers of these regions were ready to adopt the contract farming of *Artemisia annua*. More than 100 farmers entered into the agreement with IPCA Laboratories, Ratlam., M.P.

Survey of Organic tulsi belt of Rath, Hamirpur. Uttar Pradesh, 17 December 2010

CIMAP has undertaken the survey of organic tulsi growing areas of Umaria, Rath, Hamirpur and interacted with the farmers. The farmers were apprised about the improved technology for cultivation of *Ocimum.* The Farmers were told to adopt other medicinal and aromatic crops with the technical guidance of CIMAP.

MAPs Awareness meets organized

Baruasagar, Jhansi.U.P, 7 September 2010

More than 150 farmers participated in the gosthi. Lectures and demonstration of lemongrass, palmarosa, Basil, Ashwagandha, Kalmegh, Garanium and Serpgandha were given by Dr. VKS Tomar, Dr. Alok Krishna and Shri RP Yadav. Some of the farmers, who have grown Krishna variety of lemongrass, were also honoured. CIMAP has developed Self Help Group of the farmers and possibility of setting up of distillation facility from DBT sponsored project is being explored for the next year.

Niwari, Tikamgarh, M.P., 4 December 2010

More than 125 farmers including agriculture students and faculty members participated in the gosthi. Lectures and demonstration of lemongrass, palmarosa, Basil, Aswagandha, Kalmegh and Serpgandha were given by Dr. VKS Tomar and Dr. Alok Krishna.

Rath, Hamirpur, U.P., 23 January 2011

About 100 farmers participated in the MAPs awareness gosthi. Some of the farmers were interested in cultivation of those plant which are suitable for orchards. Dr. Alok Krishna delivered lecture on Basil, Lemongrass, Palmarosa and Kalmegh. Small and marginal farmers were advised to adopt Tulsi cultivation in the region. About 50 growers of organic Tulsi were also participated in the gosthi. CIMAP would like to undertake organic cultivation of Tulsi for its oils amongst the small and marginal farmers of Hamirpur.

Crop-economics surveys

Survey for collection of data for working out the crop economics was conducted during the months of April, May, September and October 2010 in Sitapur, Barabanki, Badaun and Raebareli districts of Uttar Pradesh. The data were collected from more than 100 farmers' fields in respect of different aromatic and medicinal crops such as Mint, Khus, Tulsi and Satavar. It was found that aromatic crops gave more income as compared to other crops grown in these districts. Sataver was found to be an upcoming new crop in the farmers' field in Sitapur and Raebareli districts. It was found that in Badaun district, area under mint cultivation decreased drastically (by up to 40%) due to depletion in the water table and sudden increase in temperature during the month of March-April, 2010.

Visitors to CSIR-CIMAP technology window

More than 5000 visitors including farmers, students, entrepreneurs, government officials, representatives of industries, NGOs and common people visited CSIR-CIMAP technology window and gathered knowledge about different aspects of medicinal and aromatic plants.

Women Entrepreneurial Training Facility (WETF)

CSIR-CIMAP has set up a Women Entrepreneurial Training Facility (WETF) for providing regular technical guidance to women at Chandrika Devi Tample, Near Bakshi-ka-Talab. The WETF was inaugurated by Prof. Samir K. Brahmachari, DG, CSIR on 29th March, 2011. To further sustain the activity, two self help groups (SHGs) have been established, one each near Chandrika Devi Temple and Dewa Sharief Dargah. This has directly benefited about 75 '*Prasad*' selling families residing in the villages near the vicinity of worship place who are making and selling the agarbattis in their own shop. The CSIR-CIMAP innovation based on use of offered flowers for making incense sticks is proving to be a replicable model for sustainable livelihood of poor women in rural areas.

Women group making incense sticks in WETF

WETF set up by CSIR-CIMAP

11. INFORMATION AND PROJECT MANAGEMENT

11.1. Comprehensive Traditional Knowledge Digital Library (Rakesh Tiwari, G.D. Bagchi, Manoj Semwal, Amit Mohan, Sanjay Singh)

Data collection, validation and entry of pharmacognostical information, uses and bibliographic references of 917 plants was completed, entered in the database was sent to the CTKDL unit, wherein, the data from various participating laboratories is collated and put in the portal for verification of the patents being filed and to stop the misuse of the traditional knowledge exiting. The data entered in the database is about the part of plant used for medicinal properties, pharmacology, organoleptic characters, external and internal morphology.

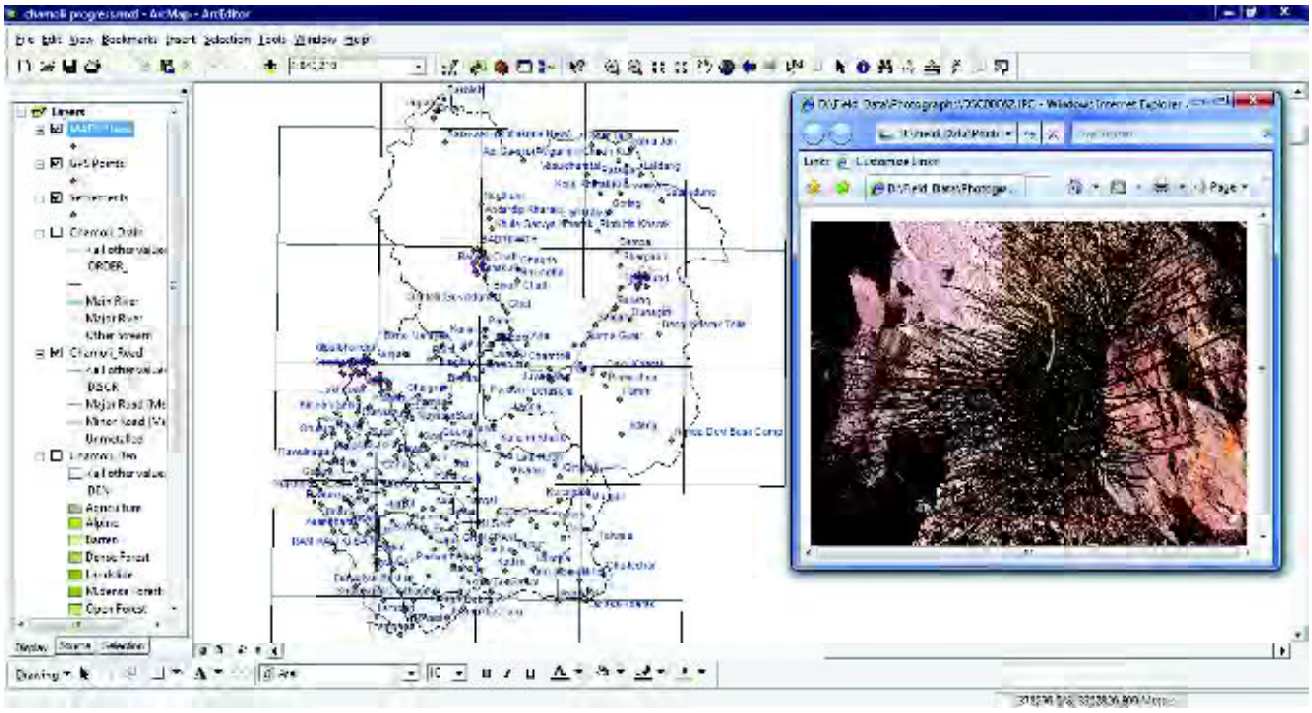
11.2. SMS-based agro-advisory services for medicinal and aromatic plant (MAPs) growers (Alok Kalra, Rakesh Tiwari, Manoj Semwal, Amit Mohan, Sanjay Singh)

SMS based agro-advisory application for the medicinal and aromatic plant growers was developed to disseminate knowledge about MAP's elite varieties, good agricultural practices, diseases, market rates and harvesting time. The service was launched, during the Farmer's Fair (Kisan Mela) held at CIMAP on 31st Jan 2011 in Lucknow. More than 500 farmers registered on first day itself. The SMS based agro-advisory services are bilingual – English and Hindi. It can be also customized to local regional languages.

11.3. GIS studies on the cultivation and conservation status of medicinal and aromatic plants (MAPs) of Uttarakhand (Manoj Semwal, Rakesh Tiwari, Amit Mohan, Sanjay Singh)

Development of bio-geographical database of selected medicinal and aromatic plants of Uttarakhand was initiated. A field survey was conducted for the districts Chamoli and Udham Singh Nagar districts of Uttarakhand to collect the GPS locations and population studies. The Geographical Information System (GIS) data layers like land use, forest type were generated for the districts and overlayed along with the GPS locations of the medicinal and aromatic plants of the district.

The bio-geographical database of Chamoli District for the rare and threatened medicinal plants *Allium stracheyi*, *Ephedra gerardiana*, *Angelica glauca*, *Aconitum balfourii*, *Aconitum heterophyllum*, *Aconitum violaceum, Berberis aristata* and *Bergenia stracheyi* was developed. The GIS database for the cultivated medicinal and aromatic plants (*Artemisia annua, Asparagus racemosus* (Satavar), *Citronella* spp., Lemon grass and *Mentha citrata)* was developed for Udham Singh Nagar district.



Biogeographical database for Chamoli District, Uttarakhand

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PLANT VARIETIES RELEASED

Plant Name	Variety Name	Release Date	Nodal Scientist	DUS Characters
Menthofuran rich mint *(Mentha piperita* L.*)*	CIMAP-Patra	25.9.2010	Birendra Kumar	Erect, non-flowering, propagule formation-sucker, Plant height- 50-65cm, Leaf length-4.86-5.93 cm, Leaf width-2.93-3.53 cm, Leaf:Stem ratio-0.87-1.22, Oil content-0.25-0.35%, Menthofuran content-35.0-46.0%, Oil yield- 60-65 kg/ha
Stevia *(Stevia rebaudiana* Bert.)	CIMAP-Madhu	29.1.2011	RK Lal	Closed, medium and dark green leaves, Stem colour-dark green, Fresh leaf yield-133.77 q/ha, Dry leaf yield- 43.05 q/ha, Stevioside content-12.57%, Stevioside-513.14 kg/ha, Rebaudioside content-5.80%, Rebaudioside-249.69 kg/ha, Dulcoside content-0.20%
Opium poppy (*Papaver somniferum* L.)	CIMAP-Ajay	29.1.2011	RK Lal	Open, Stem Colour-Whitish green, Colour of seed-White, Plant height-110 cm, Days to flower (50%)-70 days, Capsule Nos./Plant-5.00, Peduncle length-30cm, Maturity-120 days, Seed yield-9.50 q/ha, Straw yield-8.50 q/ha, Total poppy concentrate in straw-1.70 kg/ha, *Morphine-1.0235%, *Codeine-0.0550%, *Thebaine-0.2100%, *Nacrotine-6.200%, *Papavarine-0.400% *in straw concentrate

PATENTS GRANTED

SN	Title	Inventors	Country	Patent Number	Grant Date
1	Antimicrobial and anticancer properties of methyl beta orcinolcarboxylate from lichen (*Everniastrum cirrhatum)*	SPS Khanuja, TRS Kumar, VK Gupta, Preeti Chand, Ankur Garg, SK Srivastava, SC Verma, D Saikia, MP Darokar, AK Shasany, Anirban Pal	Japan	4498930	23.4.2010
2	Alpha arteether resistance domain	SPS Khanuja, Suchi Srivastava, AK Shasany, TRS Kumar, MP Darokar, Preeti Chand, Sushil Kumar	India	240876	8.6.2010
3	Stable high ginsenoside yielding callus line of *Panax quinquefolium* (American ginseng) and a method for developing such stable high ginsenoside yielding callus line	Archana Mathur, AK Mathur, GC Uniyal, Mahesh Pal, RS Sangwan	Japan	4545255	9.7.2010
4	A process for one pot conversion of artemisinin into artesunic acid and 10-esters of DHA	RS Bhakuni, Tarun Singh, AP Kahol, SPS Khanuja	Canada	2508333	20.7.2010
5	A novel anticancer activity of loganin and its potential synthetic analogues	SPS Khanuja, SK Srivastava, Ankur Garg, Merajuddin Khan, MP Darokar, Anirban Pal	USA	7767798	3.8.2010

6	A process for the production of an anticancer compound (-)3, 4-divanillyltetrahydrofuran from (-) secoisolariciresinol isolated from the plant *Taxas wallichiana*	SK Chattopadhyay, Sachin Srivastava, Vinayak Tripathi	India	242165	17.8.2010
7	A process for the production of red dye from the plant *Terminalia muelleri*	MM Gupta, DV Singh, RK Verma, SC Singh	India	242327	24.8.2010
8	Unique DNA marker for tagging high artemisinin yield in *Artemisia annua* and use of method to screen high yielding plants	SPS Khanuja, Shilpi Paul, AK Shasany, MP Darokar, AK Shukla, MM Gupta, Anuruddha Kumar	China	ZL200380110931.7	8.9.2010
9	Use of a natural compound as bio-enhancer of anti-infective agents	SPS Khanuja, JS Arya, SK Srivastava, AK Shasany, TRS Kumar, MP Darokar, Sushil Kumar	Canada	2441629	21.9.2010
10	Formulation useful as a nitrification and urease inhibitor and a method of producing the same	DD Patra, Usha Kiran, M Anwar, S Chand, Sushil Kumar	Brazil	PI0006931.0	21.9.2010
11	Primer composition and a kit useful for the identification of *Phyllanthus* species	AK Shasany, Neeraj Jain, SPS Khanuja, MP Darokar, AK Gupta, V Sundaresan, GD Bagchi, SC Singh, Janardan Singh, SP Jain, RR Rao	USA	7829692	9.11.2010
12	Pharmaceutical composition containing cow urine distillate and an antibiotic .	SPS Khanuja, Sushil Kumar, AK Shasany, JS Arya, MP Darokar, Monika Singh, Prachi Sinha, Soumya Awasthi, SC Gupta, VK Gupta, MM Gupta, RK Verma, Sweta Agarwal, SB Mansinghka, SH Dawle	Canada	2425025	23.11.2010
13	A process for regioselective demethylation of p-methoxy group in phenolic ester and diaryl ketone moieties	AS Negi, SK Chattopadhyay, Sachin Srivastava, AK Bhattacharya	India	244222	24.11.2010
14	A novel plant growth promoting naphthophenone derivative from gallic acid	SPS Khanuja , MP Darokar, Ankur Garg, T Padmapriya, AK Shasany , A S Negi, SK Chattopadhyay, Sachin Srivastava, AK Bhattacharya	Australia	2003290421	9.12.2010
15	An improved process for analytical and quantitative isolation of withaferin-A from plant materials and products therefrom	RS Sangwan, ND Chaurasiya, LN Mishra, Payare Lal, GC Uniyal, NS Sangwan, AK Srivastava, KA Suri, GN Qazi, Rakesh Tuli	India	244612	13.12.2010
16	A new nitrile glycoside useful as a bioenhancer of drugs and nutrients and the process of its isolation from *Moringa oleifera*	SPS Khanuja, JS Arya TRS Kumar, D Saikia Harpreet Kaur, Monika Singh SC Gupta, AK Shasany MP Darokar, SK Srivastava MM Gupta, SC Verma Anirban Pal	Japan Canada	4652820 2520902	24.12.2010 25.1.2011

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Singh Tula
Suresh TP
Valmiki Harpal
Verma Nirmala
Verma RY

CIMAP BUDGET AT A GLANCE

	Rupees in Lakhs
Pay & Allowances	2109.713
Contingencies (P-04)	215.504
H.R.D. (P-05)	3.829
Lab Maintenance (P-06)	110.087
Staff QRS, Maintenance (P-701)	20.596
Chemicals/Consumables & Other Research Expenditures (P-07)	355.620
Works & Services (P-50)	88.309
Apparatus & Equipments - Scientific (P-50)	399.712
Office Equipments (P-50)	4.511
Furniture & Fittings (P-50)	5.546
Library Books (P-50)	3.673
Library Journals (P-50)	93.266
Staff Qtrs. (Construction) (P-702)	15.001
CSIR Network Projects (NWP)	569.719
Total	3766.205
Pension (P804)	750.480
EMR (P81)	154.891
External Budgetary Resource	
Lab Reserve Fund (LRF)	73.084
External Cash Flow (ECF)	329.572

Prof. Samir K Brahmachari, DG-CSIR unveiling the plaque of Women Entrepreneurial Training Facility (WETF) set up by CSIR-CIMAP at Chandrika Devi Temple, Bakhshi-ka-Talab, (Near Lucknow), Uttar Pradesh

Director CSIR-CIMAP Prof. Ram Rajasekharan holding India Agri Award Trophy
The award was sponsored by Mahindra Group and Zee TV

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