RESEARCH PROJECT PROPOSAL

ON

INVESTIGATIONS ON POTENTIAL OF BRASSINOSTEROIDS IN ACCELERATING METABOLISM OF PESTICIDES IN VEGETABLES

SUBMITTED TO

THE DEPARTMENT OF BIOTECHNOLOGY
MINISTRY OF SCIENCE & TECHNOLOGY, GOVERNMENT OF INDIA
BLOCK-2, 6-8 FLOORS, C.G.O. COMPLEX, LODHI ROAD, NEW DELHI

INDIAN INSTITUTE OF HORTICULTURAL RESEARCH,
HESSARAGHATTA LAKE P.O., BANGALORE - 560089
12. Scope of application indicating anticipated product and processes

The project envisages to develop a technique for degrading pesticide residues within the crop to harmless substances. Adoption of this technology will significantly reduce the environmental hazards posed through the use of chemical pesticides through use of safe, natural substances.

The other significant outcome of the project will be understanding role of brassinosteroids on major detoxification enzymes present in plant. Also, this is a basic study which will explore the possibility of using BRs for food and environmental safety.
Public concern over high levels of toxic pesticides residues in food is increasing every day. Safety is an important food quality parameter for the consumer and an important aspect of food safety is control of pesticide residues in food. It is even more important to ensure safety in horticultural produce such as fruits and vegetables as these are consumed within a short period after harvest and also often consumed in raw form. There are several measures to minimize pesticide residues in the produce at harvest, but, these are rarely followed, rather, due to indiscriminate and non recommended use of pesticides harmful pesticide residues remain on the produce.

Brassinosteroids (BRs) are a class of naturally occurring plant polyhydroxysteroids which have been recognized as a new kind of phyto-hormone that play a vital role in plant growth and development. More than 40 related compounds have been isolated, of which three -- brassinolide, 24-epibrassinolide and 28-homobrassinolide-are biologically active at levels as low as 0.005 µg/ml. The growth regulatory activity of BRs is suggested to be the result of their influence on the metabolic process associated with photosynthesis, nucleic acid and protein synthesis. In addition, BRs have been shown to counteract abiotic stress effects such as chilling, drought, salinity and heat stress in plants. There are also reports that BRs are effective in reducing damage caused by pesticides (Xia et al, 2006).

In a recent report, BRs have been shown to accelerate metabolism of pesticides in plant and as a consequence reduce their residue level (Xia et al, 2009) and this effect was associated with enhanced expression of detoxification genes. Thus, BRs may be promising, environment friendly natural substances which can be used to reduce risk of human exposure to pesticides. However, no systematic studies have been carried out to demonstrate the effect of BRs on pesticides at actual levels of pesticide application, and more importantly their effect on detoxification of the pesticides in the field, at harvest.

The present project proposal therefore envisages to explore if BRs could be used to degrade pesticides to harmless products within the plant after its intended use i.e. control of target pest, was achieved. We are aware of the enzymes used by plants to detoxify xenobiotics including pesticides. The effect of BR treatment on some of these enzymes viz. P450 monooxygenase, glutathione S-transferase (GST), peroxidase (POD) and UDP-β-glycosyltransferase (UGT) will also be studied to determine the relationship between the BRs and detoxification enzymes present in plant.
PART II: PARTICULARS OF INVESTIGATORS

Inclusion of co-investigator(s) is mandatory for investigators retiring before completion of the project.

14. Principal Investigator:

Name: Debi Sharma  
Date of Birth: 11/07/1961  
Sex (M/F): F  
Designation: Principal Scientist  
Department: Pesticide Residue Laboratory, Division of Soil Science & Agricultural Chemistry  
Institute/University: Indian Institute Of Horticultural Research  
Address: IIHR, Hessraghatta Lake Post, Bangalore PIN- 560089  
Telephone: 080-28466420 extn 237, Fax: 080-28466291  
E-mail: dsharma@iihr.ernet.in, debisharma@gmail.com

Number of research projects being handled at present: 3

Co-Investigator

15. Name: Dr. K.K. Upreti
Date of Birth: 02.10/1960  
Sex (M/F): M  
Designation: Principal Scientist  
Department: Division of Plant Physiology and Biochemistry  
Institute/University: Indian Institute Of Horticultural Research  
Address: IIHR, Hessraghatta Lake Post, Bangalore PIN- 560089  
Telephone: 080-28466420 extn 237 Fax:080-28466291  
E-mail: kku@iihr.ernet.in

Number of Research projects being handled at present: 3
16. Introduction (not to exceed 2 pages or 1000 words)
Pesticides are used to control pests of crops and their use has played a major role in increasing the availability of produce to the consumer as they have allowed growers and handlers to increase production, extend shelf life and improve the appearance of many foods. However, high levels of pesticides residues in food are a matter of concern especially in horticultural produce such as fruits and vegetables as these can be hazardous to human health. The matter has been debated and discussed in several forums. Since, human safety is the foremost consideration in food production, superseding even the obvious importance of economic factors, international systems of legal control have been established to prevent residue contaminated products from entering the human food supply. In this way, an important aspect of food safety is the control of pesticide residues in food. Public concern over pesticide residues in food is increasing and it has become a significant food safety concern. Safety of the food is ensured in exported fruits and vegetables by stringent monitoring of the produce for pesticide residues among other parameters, however, the produce meant for domestic market is usually not monitored. There are several measures to minimize pesticide residues in the produce at harvest, viz. following safe waiting periods before harvest, integrated pest management practices, reducing applications of chemical pesticides at near harvest stages of crop growth etc. However, farmers want to maximize their yield and in their anxiety to save their crop often do not follow these measures. Degradation of a pesticide with time to non toxic substances depends on its chemical nature and in most of the cases, the produce has to be harvested before the pesticide has degraded completely resulting in harmful residues of pesticides on the produce.

16.1 Origin of the proposal
It would be an ideal situation if a pesticide could be degraded quickly to harmless products after its intended use i.e. control of target pest, was achieved. Thus, accelerated metabolism of the pesticide within the plant before harvest would solve the problem of harmful pesticide residues in the produce at harvest. This type of accelerated metabolism of pesticides has scarcely been studied. Brassinosteroids (BR), are naturally occurring compounds, which have an essential role in plant growth and development, these are also involved in plant responses to environmental stress and plant defense against various pathogens. Little is, however, known about involvement of brassinosteroids in plant responses to organic pollutants such as pesticides. Only one study points to faster reduction in pesticide residues if the plants were pretreated with BR. Plants are able to transform/detoxify pesticides and other xenobiotics using a phased detoxification system. Phase I (activation) reactions which usually involve hydrolysis or oxidation and phase II (conjugation) reactions, which are involved in synthesis. Absorbed pesticides are first metabolically activated by "phase I" enzymes, such as P450 monooxygenase, peroxidase and carboxylesterases. The second phase involves conjugation to glutathione (GSH) and glucose catalyzed by glutathione S-transferase (GST) and UDP-glycosyltransferase (UGT), respectively. The third phase of pesticide metabolism is sequestration and storage of soluble metabolites either in vacuoles or in apoplast. The glutathione S-conjugates are actively transported to the vacuoles or apoplast by ATP - dependent membrane pumps. Effects of pesticides on enzymes involved in detoxification pathways can be determined with and without application of brassinosteroids to determine if the activities of these enzymes are influenced by
One study has shown that a pesticide viz. chlorpyrifos induced activities of enzymes GST, peroxidase (POD) and glutathione reductase (GR) were higher in BR-pretreated cucumber plants than in non-pretreated plants. There is therefore a need to study the relationship between detoxification enzyme activity and pesticide application with and without BR.

16.2 . (a) **Rationale of the study supported by cited literature** - It has been shown that application of brassinosteroids can accelerate metabolism of various pesticides in plants and consequently reduce their residue levels in plants. In a recent study a brassinosteroid was shown to accelerate the degradation of pesticides and this effect was associated with enhanced expression of detoxification genes. However, hardly any systematic studies have been carried out to demonstrate the effect of BRs on pesticides at actual levels of pesticide application, and more importantly their effect on detoxification of the pesticides at harvest.

(b)**Hypothesis** – Brassinosteroids can be used to reduce the levels of pesticide residues in horticultural produce just prior to their harvest to below permissible levels (MRLs) of the respective pesticides.

(c) **Key questions** – (1) Do brassinosteroids actually accelerate metabolism of pesticides in plants? If yes, at what stage of crop growth and at what application concentration? (2) To what extent can the pesticide residue load in fruits and vegetables be reduced at harvest by application of such phytochemicals? (3) What is the mechanism by which brassinosteroids detoxify pesticidal xenobiotics? (4) Are the activities of detoxification enzymes in plants influenced by BR treatment?

16.5 **Current status of research and development in the subject (both international and national status)**

**International** – A large amount of information is available about the role of brassinosteroids in plant growth and development, specifically, promotion of vascular differentiation, pollen elongation etc. (Clouse and Sasse, 1998). BRs are also implicated in plant responses to abiotic environmental stresses and to undergo profound changes in plants interacting with bacterial, fungal and viral pathogens (Bajguz and Hayat, 2009). However, only one study (Xia Xiao Jian et al. 2009) has so far been carried out to show that pretreatment with a brassinosteroid resulted in significant reductions in residues of the pesticide chlorpyrifos, in cucumber and also alleviated the toxic effect of chlorpyrifos on photosynthesis. The rate at which the pesticide was treated is however not clear.

**National** – No studies have been carried out in the area in India.

16.6 **The relevance and expected outcome of the proposed study**
The proposed study is extremely relevant in the present context as consumer awareness and preference for safe food is increasing in India. The scope of organic farming in producing safe food is limited and therefore studies towards development of a safe and reliable technique to detoxify pesticides in plants before harvest is urgently required. The outcome of the present
The study will clearly bring out the role (if any) of brassinosteroids in acceleration of pesticide metabolism in plants and therefore their utility in addressing food safety concerns.

16.7 Preliminary work done so far

Two of the common pesticides used in production of vegetables have been identified and residue analysis methods for determination of the parent molecules as well as the metabolites have been standardized.

References

17. Specific objectives (should be written in bulleted form, a short paragraph indicating the methods to be followed for achieving the objective and verifiable indicators of progress should follow each specific objective)

- To investigate the role of brassinosteroids in reducing the load of pesticide residues in selected vegetables at harvest.

Two vegetables, (i) tomato in open field and (ii) capsicum in polyhouse will be raised. Brassinosteroids and recommended pesticides will be sprayed on the crop as per technical programme. Residue analysis of pesticides will be carried out using standardized protocols utilising GC, GC-MS and HPLC equipments. The experiments will be repeated for two seasons for confirmation. Verifiable indicator of progress — Pesticide residue levels in the vegetables with and without brassinosteroid application.

- To investigate the role of brassinosteroids on the activities of enzymes involved in detoxification of pesticides.

Activity of enzymes, P450 monooxygenase, glutathione S-transferase (GST), peroxidase (POD) and UDP-β-glycosyltransferase (UGT) will be determined in plant tissues using spectrophotometric methods. Verifiable indicator of progress — Activity of enzymes following application of pesticides and pesticides + brassinosteroid.
18.1 Work plan (methodology/experimental design to accomplish the stated aim)

Effect of brassinosteroids in reducing pesticide residues in vegetables

Greenhouse cultivation of vegetables especially off season and exotic vegetables is gaining importance, therefore, two vegetable crops, viz. tomato in open field and capsicum (bell pepper) in polyhouse will be grown for two seasons each. Effect of two different concentrations of two commercially available brassinosteroids on residues of the two pesticides and their toxic metabolites will be assessed. The pesticides chosen tentatively are imidacloprid and fipronil, which are used extensively on these crops even at near harvest stages. These are persistent pesticides with half life of 10 days or more and their residues pose human health hazard. Also both these pesticides degrade to metabolites some of which are also toxic. Therefore, fate of the parent molecule as well as toxic metabolites will be determined. The brassinosteroids will be assessed as post pesticide application treatments at fruit formation stage of the respective crop. Residues of pesticides in tomato and capsicum fruits will be analysed by standard analytical protocols using GC and HPLC equipments.

Role of brassinosteroids on the activity of detoxification enzymes

Detoxification enzymes viz. P450 monooxygenase, glutathione S-transferase (GST), peroxidase (POD) and UDP glycosyltransferase (UGT) will be determined prior to and after each brassinosteroid treatment, in tissues of tomato and capsicum plants by spectrophotometric methods using standard protocols. Relation between enzyme activity and metabolic degradation of pesticides will also be studied.

18.2 Connectivity of the participating institutions and investigators (in case of multiinstitutional projects only)

Not Applicable

18.3 Alternate strategies (if the proposed experimental design or method does not work what is the alternate strategy)

The work proposed is important basic work which attempts to solve a much debated issue of pesticide residues in food in general and in vegetables in particular. In case the strategy fails, other safe plant growth regulating compounds will be explored for the same purpose.
19. Timelines:

<table>
<thead>
<tr>
<th>Period of study</th>
<th>Achievable Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Months</td>
<td>Raising of crop, purchase of equipments, procurement of standards</td>
</tr>
<tr>
<td>12 Months</td>
<td>Residues of imidacloprid and fipronil in tomato (season 1) with and without BR treatment</td>
</tr>
<tr>
<td>18 Months</td>
<td>Residues of imidacloprid and fipronil in capsicum (season 1) with and without BR treatment. Effect of BR treatment on enzyme activity in tomato.</td>
</tr>
<tr>
<td>24 Months</td>
<td>Residues of imidacloprid and fipronil in tomato (season 2) with and without BR treatment. Effect of BR treatment on enzyme activity in capsicum.</td>
</tr>
<tr>
<td>30 Months</td>
<td>Residues of imidacloprid and fipronil in capsicum (season 2) with and without BR treatment.</td>
</tr>
<tr>
<td>36 Months</td>
<td>Gap analysis, compilation and analysis of data, report writing.</td>
</tr>
</tbody>
</table>

20. Name and address of 5 experts in the field

<table>
<thead>
<tr>
<th>SNo</th>
<th>Name</th>
<th>Designation</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dr. V.T. Gajbhiye</td>
<td>Head, Division of Agricultural Chemicals</td>
<td>Division of Agricultural Chemicals, IARI, New Delhi-110012&lt;br&gt;Email: <a href="mailto:vtgajbhiye2@yahoo.co.in">vtgajbhiye2@yahoo.co.in</a>&lt;br&gt;Tel: 011-25843272</td>
</tr>
<tr>
<td>2.</td>
<td>Dr. Prem Dureja</td>
<td>Emeritus Scientist</td>
<td>Division of Agricultural Chemicals, IARI, New Delhi-110012&lt;br&gt;Email:<a href="mailto:pd_dureja@yahoo.com">pd_dureja@yahoo.com</a></td>
</tr>
<tr>
<td>3.</td>
<td>Dr. M.D. Awasthi</td>
<td>Ex-Head of Division, Division of Soil Science &amp; Agricultural Chemistry</td>
<td># 47, 3rd Cross, KEB Layout, Sanjaynagar, Bangalore-560094&lt;br&gt;Email :<a href="mailto:mdawasthi@yahoo.com">mdawasthi@yahoo.com</a>&lt;br&gt;Tel : 080-23417644</td>
</tr>
<tr>
<td>4.</td>
<td>Dr. L.P. Srivastava</td>
<td>Senior Principal Scientist</td>
<td>Indian Institute of Toxicology Research&lt;br&gt;P.O. Box 80, M.G. Marg&lt;br&gt;Lucknow-226001&lt;br&gt;Email: <a href="mailto:lpsrivastava@iitr.res.in">lpsrivastava@iitr.res.in</a>&lt;br&gt;Tel: 0522-2620106</td>
</tr>
<tr>
<td>5.</td>
<td>Dr. Mukul Das</td>
<td>Chief Scientist</td>
<td>Indian Institute of Toxicology Research&lt;br&gt;P.O. Box 80, M.G. Marg&lt;br&gt;Lucknow-226001&lt;br&gt;Email: <a href="mailto:mukul@iitr.res.in">mukul@iitr.res.in</a>&lt;br&gt;Tel: 0522-2963826</td>
</tr>
</tbody>
</table>
**PART IV: BUDGET PARTICULARS**

### A. Non-Recurring (e.g. equipments, accessories, etc.)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Item</th>
<th>Year 1 (Rs)</th>
<th>Year 2 (Rs)</th>
<th>Year 3 (Rs)</th>
<th>Total (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rotary vacuum evaporator</td>
<td>3,50,000</td>
<td></td>
<td></td>
<td>3,50,000</td>
</tr>
<tr>
<td>2.</td>
<td>Refrigerated centrifuge</td>
<td>3,00,000</td>
<td></td>
<td></td>
<td>3,00,000</td>
</tr>
</tbody>
</table>

**Sub-Total (A) = Rs. 6,50,000/-**

**Justification (Equipments):** The rotary vacuum evaporator in the laboratory is more than 25 years old and is used continuously, a replacement is therefore, urgently required for more efficient and faster concentration of samples. A table top refrigerated centrifuge is required for enzyme studies.

### B. Recurring

#### B.1 Manpower (See guidelines at Annexure-III)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Position</th>
<th>No</th>
<th>Consolidated Emolument (Rs.)</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>JRF</td>
<td>1</td>
<td>18200 PM for Year 1 &amp; 2, 20,800PM for Year 3</td>
<td>218400</td>
<td>218400</td>
<td>249600</td>
<td>6,86,400</td>
</tr>
<tr>
<td>2.</td>
<td>Skilled Assistant</td>
<td>1</td>
<td>8000 PM</td>
<td>96,000</td>
<td>96,000</td>
<td>96,000</td>
<td>2,88,000</td>
</tr>
</tbody>
</table>

**Sub-Total (B.1) = Rs. 9,74,400/-**

**Justification (Manpower):** There is requirement of 1 JRF for undertaking analytical work in two laboratories as well as in field and polyhouse. The work also requires a Skilled Assistant, a science graduate, who can assist in sample preparation and estimation, and also supervise field and polyhouse trials.

#### B.2 Consumables

<table>
<thead>
<tr>
<th>S.No</th>
<th>Item</th>
<th>Quantity</th>
<th>Year 1 (Rs)</th>
<th>Year 2 (Rs)</th>
<th>Year 3 (Rs)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chemicals, glassware, plasticware, pesticide standards, gas, SPE cartridges, glassware, GC/HPLC consumables e.g. columns, septa, ferrules, seed, spray chemicals etc.,</td>
<td></td>
<td>2,50,000</td>
<td>2,50,000</td>
<td>2,50,000</td>
<td>7,50,000</td>
</tr>
</tbody>
</table>

**Sub-Total (B.2) = Rs. 7,50,000/-**

**Justification (Consumables):** There is requirement of a lot of consumables for sample preparation, analytical determination etc. in the laboratory and some inputs for the farm and polyhouse.
<table>
<thead>
<tr>
<th>Item</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.3 Travel</td>
<td>1,00,000</td>
<td>1,00,000</td>
<td>50,000</td>
<td>2,50,000</td>
</tr>
<tr>
<td>B.4 Contingency</td>
<td>50,000</td>
<td>50,000</td>
<td>50,000</td>
<td>1,50,000</td>
</tr>
<tr>
<td>B.5 Overhead (If applicable)</td>
<td>1,00,000</td>
<td>1,00,000</td>
<td>50,000</td>
<td>2,50,000</td>
</tr>
<tr>
<td>Sub-total of B</td>
<td>8,14,400</td>
<td>8,14,400</td>
<td>7,45,600</td>
<td>23,74,400</td>
</tr>
<tr>
<td>(B.1+B.2+B.3+B.4+B.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand Total (A + B)</td>
<td>14,64,400</td>
<td>8,14,400</td>
<td>8,14,400</td>
<td>30,93,200</td>
</tr>
</tbody>
</table>

**Justification (other items):** There is requirement of TA for two scientists, 1 JRF, contingency for hiring casual labour for the period required and overhead for meeting institutional charges.

(Note: Please give justification for each head and sub-head separately mentioned in the above table.
Financial Year: April - March
In case of multi-institutional project, the budget estimate to be given separately for each institution)

**PART V : EXISTING FACILITIES**

Resources and additional information

1. Laboratory:
   a. Manpower
      NIL
   b. Equipments
      - Gas liquid chromatograph
      - High Performance liquid chromatograph
      - UV-VIS Spectrophotometer
      - SPE Vacuum manifold
      - Deep Fridge (-20°C)
      - Orbital Shaker
      - Fridge

2. Other resources such as clinical material, animal house facility, glass house. Experimental garden, pilot plant facility etc.

   - Field plot and polyhouse facilities
PART VI: DECLARATION/CERTIFICATION

It is certified that

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.
b) the same project proposal has not been submitted to any other agency for financial support.
c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)
d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.
e) if the project involves the utilisation of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.
f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.
g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Deptt. of Expenditure, as contained in Annexure-V.
h) we agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.
i) the institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.
j) the Institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Executive Authority of Institute/University with seal : (Dr. Amrik Singh Sidhu)
Date :

Signature of Principal Investigator : (Dr. Debi Sharma)
Date :

Signature of Co-Investigator : (Dr. K.K. Upreti)
Date :
PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS

Provide the following information for the key personnel in the order listed on PART II. Follow this format for each person. DO NOT EXCEED THREE PAGES

Principal Investigator
Name: Dr. Debi Sharma
Designation: Principal Scientist
Department/Institute/University: Indian Institute Of Horticultural Research
Date of Birth: 11.07.1961   Sex (M/F)  F   SC/ST : .No

Education (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Institution, Place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Delhi University, Delhi</td>
<td>M.Sc. Chemistry</td>
<td>1984</td>
<td>Chemistry with specialization in Organic Chemistry</td>
</tr>
<tr>
<td>2.</td>
<td>IARI, New Delhi</td>
<td>Ph.D</td>
<td>1989</td>
<td>Agrochemicals and Agricultural Chemistry</td>
</tr>
</tbody>
</table>

A. Position and Honors

Position and Employment (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Institution, Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IIHR, Bangalore</td>
<td>Principal Scientist</td>
<td>27.7.2006</td>
<td>Till Date</td>
</tr>
<tr>
<td>2.</td>
<td>IIHR, Bangalore</td>
<td>Senior Scientist</td>
<td>27.7.1998</td>
<td>26.7.2006</td>
</tr>
<tr>
<td>3.</td>
<td>IIHR, Bangalore</td>
<td>Scientist(Sr. Scale)</td>
<td>06.06.1994</td>
<td>26.7.1998</td>
</tr>
<tr>
<td>4.</td>
<td>IIHR, Bangalore</td>
<td>Scientist</td>
<td>06.06.1989</td>
<td>05.06.1994</td>
</tr>
<tr>
<td>5.</td>
<td>Delhi University</td>
<td>Lecturer</td>
<td>21.01.1989</td>
<td>10.05.1989</td>
</tr>
</tbody>
</table>

Honors/Awards

1. Selected to the Bureau of Indian Standards (Govt of India) Sectional Committee no. FAD 34 (Now FAD 1) on pesticide residues as member from IIHR from 1999 till date.
3. Awarded Research Scholarship for a project Persistence and dissipation of different formulations of imidacloprid in okra plant and soil" from ASPEE Agricultural Research and Development Foundation Malad, Mumbai.
5. IARI Senior Research Fellowship for Doctoral Research Work

Professional Experience and Training relevant to the Project
Worked for past 22 years in the field of analysis of pesticide residues in horticultural produce and have gained technical expertise in different aspects of the field and contributed to the

- determination of persistence pattern of pesticide residues in vegetables and fruits for establishment of pre harvest intervals and MRL in open field and in polyhouse.
- pesticide residue analytical protocol standardizations by GLC, HPLC, GC-MS. A modified more efficient method of HPLC analysis of carbendazim residues was developed which has been accepted by the Bureau of Indian Standards and is in use by various laboratories in the country
- multi pesticide residue analysis method standardizations of pesticides in fruits, vegetables and soil.
- mobility of pesticides in soil and uptake of soil applied pesticides into plant.
- development of immunoassay techniques for pesticide residue analysis.
- evaluation of suitability of post harvest pesticide treatments, pesticide treatments in IPM and IDM package development

Training undergone:

- NABL training on Laboratoy Management and Internal Audit 2-5 May, 2006, Bangalore.
- ELISA for pesticide residue analysis in foods, June 10-12, 1996, CFTRI.
- Short Course on How to develop, validate and troubleshoot GC methods Sept. 5, 2001, Bangalore.

B. Publications (Numbers only)
Patents : Nil  Others (Please specify) : Abstracts :26

Selected peer-reviewed publications (Ten best publications in chronological order)


List maximum of five recent publications relevant to the proposed area of work.


C. Research Support

<table>
<thead>
<tr>
<th>Ongoing Research Projects</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

Completed Research Projects (State only major projects of last 3 years)

NIL

Signature of Investigator : (Sd/- Debi Sharma)
Place : Bangalore
Date : 12.12.2011
Name : Dr. K.K. Upreti  
Designation : Principal Scientist  
Department/Institute/University : Indian Institute Of Horticultural Research  
Date of Birth : 02.10.1960      Sex (M/F)  M               SC/ST : No

Education  (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Institution, Place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lucknow University, Lucknow</td>
<td>M.Sc. Chemistry</td>
<td>1982</td>
<td>Chemistry with specialization in Organic Chemistry</td>
</tr>
<tr>
<td>2.</td>
<td>Indian Institute of Toxicological Research (IITR), Lucknow</td>
<td>Ph.D</td>
<td>1989</td>
<td>Chemistry</td>
</tr>
</tbody>
</table>

A. Position and Honors

Position and Employment (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Institution, Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IIHR, Bangalore</td>
<td>Principal Scientist</td>
<td>27.7.2007</td>
<td>Till Date</td>
</tr>
<tr>
<td>2.</td>
<td>IIHR, Bangalore</td>
<td>Senior Scientist</td>
<td>27.7.1998</td>
<td>26.7.2006</td>
</tr>
<tr>
<td>3.</td>
<td>IIHR, Bangalore</td>
<td>Scientist(Sr. Scale)</td>
<td>06.06.1994</td>
<td>26.7.1998</td>
</tr>
<tr>
<td>4.</td>
<td>IIHR, Bangalore</td>
<td>Scientist</td>
<td>12.06.1989</td>
<td>05.06.1994</td>
</tr>
</tbody>
</table>

Honors/Awards

- Junior/Senior Research Fellowship for Doctoral Research Work- Council of Scientific & Industrial Research (CSIR), New Delhi
- Recognized as PG Teacher, University of Horticultural Sciences, Bagalkot in the subject Plant Physiology & Biochemistry
- Recognized as PG Teacher, Andhra Horticultural University, Tadepalligudem, West Godavari Dist.
- Recognized as Research guide for guiding M.Phil & PhD students in biochemistry, Kuvempu University, Shimoga, Karnataka

Professional Experience and Training relevant to the Project

Worked for past 22 years the field of hormonal regulatory mechanism in the growth and development of horticulture crops and in abiotic stress tolerance. During this period I have contributed to different aspects of the field and gained technical expertise in the field and contributed to the

- development of ELISA procedures for plant hormones
understanding role of ABA and cytokinins and polyamines in the water stress and salinity
amelioration of stress responses by growth regulators
standardizations by HPLC polyamine and glycine betaine procedures and gibberellins by GC-MS.

B. Publications (Numbers only)
Patents : Nil  Others (Please specify) : Abstracts :39

Selected peer-reviewed publications (Ten best publications in chronological order)

List maximum of five recent publications relevant to the proposed area of work


C. Research Support
Ongoing Research Projects

<table>
<thead>
<tr>
<th>S.No</th>
<th>Title of the project</th>
<th>Funding agency</th>
<th>Amount</th>
<th>Date of Sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Understanding the mechanism of off-season flowering and fruiting in mango under different environmental conditions (Consortium Co-PI)</td>
<td>National Agriculture Innovation Project (NAIP)-ICAR, New Delhi</td>
<td>Rs 77.278 lakhs</td>
<td>Jan 21, 2009 to March 31, 2012</td>
</tr>
</tbody>
</table>

Completed Research Projects (State only major projects of last 3 years)
NIL

Signature of Investigator (Sd/- K.K.Upreti)
Place: Bangalore
Date: 12.12.2011