National Conference
on
Agrotechnology, Commerce and Sustainable Use of Medicinal and Aromatic Plants

February 6-7, 2016

Venue
National Agricultural Science Center (NASC) Complex
Todapur, Pusa Complex, New Delhi - 110012

Souvenir and Abstracts

Jointly Organised by

High Altitude Plant Physiology Research Center (HAPPRC)
H.N.B. Garhwal (Central) University
Srinagar (Garhwal), Uttarakhand

Society for Conservation and Resource Development of Medicinal Plants (SMP)
New Delhi

Sponsored by : National Medicinal Plants Board, New Delhi

OP-1

Biosafety issues related to medicinal plants: Concerns and Challenges

Monika Koul
Department of Botany, Hansraj College, University of Delhi, Delhi - 110007
E-mail : drmkoul@gmail.com

The popularity of Indian herbs is on rise and nearly half of the population all over the world relies on herbal medicines. Medicinal plants, their parts, processed products and drugs made from these are freely sold in health food shops and supermarkets all over the world. In India, herbal vendors sell these on the streets, fairs or open markets. There are no strict quality control measures adopted in the country for marketing and selling of herbal plants. The plants, their parts or products are either used externally or orally for cuing diseases. It is important that substances that are ingested should be free from any contamination. People in the country do not pay much attention to this aspect as these are mostly considered safe and free from side effects. However, over the last few years some concerns regarding contamination and adulteration have been raised. Theincreasing popularity and widespread use of their products have sparked an interest in understanding their biosafety issues. The safety and quality of raw medicinal plant materials and finished products depend on the genetic makeup of the plant, habitat conditions, collection methods, cultivation, harvest, post-harvest processing, transport systems and storage practices. Contamination by toxic substances such as high concentration of pesticides and herbicides during any of the production stages can lead to deterioration in safety and quality.

The study is undertaken to assess the heavy metal contamination in commonly consumed herbal plants and their products. Plants are being collected from various locations, from various habitats and assed for their quality parameters. It is clear from the investigations that some of the contaminants affect the bioactive constituents and result in compromise in quality. The present study is also focussed to find out at what stage the contamination is taking place so that mitigation measures are deigned to reduce the contamination.

Phytomorphology 67 (3&4) 2017, 1-11

Changes in the Leaf Epidermal Features of *Cyamopsis tetragonoloba* (L.) Taub. in Response to Lead in Soil

Monika Koul[1,*] and A.K. Bhatnagar[2]
[1]*Department of Botany, Hans Raj College, University of Delhi, Delhi-110007, India*
[2]*Indira Gandhi National Open University, Delhi-110068, India*

ABSTRACT

A pot-culture experiment was conducted to determine the effects of increased concentrations of lead on cluster bean, *Cyamposis tetragonoloba* (L.) Taub. (Fabaceae) when given in the soil in the form of lead nitrate (Pb). Seeds were grown in soil containing different levels of Pb ranging from 0 (control) to 800 μg/g soil. Pb-treated plants exhibited various toxicity symptoms; gross morphological features of the leaves and anatomical features of the plants grown were altered. Light microscopic and peel mount studies revealed changes in epidermal features of the leaf with increased Pb in the soil. Stomatal frequency increased, the size of guard cells was reduced, at high Pb levels. Trichome density was lower in leaves of Pb-treated plants compared to control plants. The percentage of abnormal stomata was 10 times higher in comparison with control at high Pb levels (600 μg/g). Leaf micro-morphological features studied by scanning electron microscopy revealed that trichomes are distorted and the cuticle covering the glandular head was disrupted so that pores in the walls underneath were exposed. At very high levels (800 μg/g), leaves showed curling, chlorosis and necrosis. These results suggest deleterious effects of metal pollutants on leaf epidermal features. The present study on cluster bean plants clearly demonstrates that the Pb in the soil is transferred from the roots right up to the minor veins of plant leaves, where epidermal features are compromised. Such compromised features can serve as indicators of the levels of pollutants in the soil.

Key words: Cluster bean, pollution, stomata, trichomes.

Abbreviations: Lead, leaf epidermis, Pb nitrate, soil, stomata, trichome

Author for correspondence: Monika Koul, e-mail: drmkoul@gmail.com

Introduction

Lead is an important metallic pollutant released from various sources, including industrial and municipal wastes, combustion of fuels and use of agrochemicals. At high concentrations in soil, it is recognized as a severe phytotoxic contaminant (Manomita et al., 2004; Opeolu et al., 2010). Disruptive changes have often been associated with plants exposed to air polluted with Pb and other heavy metals, and are considered to be indicators of the level of air pollution (Garg & Varshney, 1980; Alves et al., 2008).

Despite the reduction of Pb levels in the atmosphere on account of reduced use of Pb containing petrol, soil Pb levels have not reduced much. Atmospheric Pb is immobilized in upper layers of soil profile and is taken up by shallow rooted plants. Lead is taken up by the roots and translocated to the aerial parts of the plant where it disrupts primary and secondary metabolism (Mesmar & Jaber, 1991; Olivares, 2003). Changes are observed in plant growth, root system, leaf function and anatomical features in response to lead pollution (Eun et al., 2000; Pourrut et al., 2011). Kalat et al. (2012) observed that leaves absorb and acquire high levels of Pb and that the ability to acquire it differs with the age of the plant. Changes in biomass, shoot length, leaf area and decline in the productivity have been observed in various plants in response to Pb contaminated soils (Fragasaova, 2001; Sengar et al., 2008).

Leaf function is dependent on the anatomical and physiological coordination in the plant system. Leaf epidermal structures cuticle, epicuticular wax, stomata and pubescence contribute to the physiological and ecological functioning of the plant (Press, 1999; Tian et al., 2012). Open stomata provide a passage for water efflux by transpiration and affect other physiological features of the plant. If stomata are underdeveloped physiological processes such as ion transport are also affected. Therefore, it is important to understand the effect of lead on the leaf and its micromorphological features. Any effect on these features will be manifested in the growth and development of the plant.

Cyamopsis tetragonoloba (L.) Taub. (cluster bean), family Fabaceae, is an annual herb. It is used as fodder, green legume, or pulse for human consumption and as a source of gum ('Guar gum') for paper, textile, confectionery and pharmaceutical industries. Cluster bean is widely cultivated in the states of Haryana, Punjab and Rajasthan, where high concentrations of heavy metals, including lead as high as 118-293 mg/kg may be found in agricultural soil (Krishan & Govil; 2004, Chahal et al., 2014). Ground water samples in Rajasthan contained lead levels above the permissible limits (Duggal et al., 2014). The effects of some heavy metals have been studied on growth and other physiological processes in *Cyamopsis tetragonoloba* (El Hassan et al., 2014). However, there are no comprehensive studies on effect of the toxic heavy metals like lead on leaf micro-morphology and epidermal features. The present work was undertaken to determine the effect of soil lead on leaf features such as epidermis, stomata and trichomes, which control various fundamental physiological processes.

Material and Methods

Plant Material

Seeds of *C. tetragonoloba* (L.) Taub. var. Pusa Naveen were procured from National Seed Corporation, Pusa, New Delhi, India. Seeds of uniform size were surface sterilized with 0.001 M Mercuric chloride ($HgCl_2$) for two minutes and then thoroughly washed with deionized water.

Experimental Design

The experiment was a randomized block design with six treatments of lead nitrate having concentrations of 50, 100, 200, 400, 600 and 800 μg $Pb(NO_3)_2$ per gram soil. Pots without the treatment constituted the control set. There were five replicates for each treatment. Thus, the total number of pots was 35, each of which was filled with 6 kg soil. The soil was clayey loam at pH 7.2. Six surface-sterilized seeds were sown at a uniform distance (6 cm) in each pot. Plants were grown in a net house under 12 h light and dark cycle (25–28°C) temperature. Plants were regularly watered with tap water. Leaves were sampled from 40 day old plants. Five leaves from each plant from each of the five replicates of all the treated concentrations were randomly picked and fixed for carrying out scanning and electron microscopy.

Light Microscopy

The structure of stomata and trichomes were studied on both leaf surfaces in peel mounts prepared by treating with cupric sulphate and hydrochloric acid to easily separate both leaf surfaces (Ram & Nayyar, 1974). For calculation of the stomatal index (SI), five microscopic fields ($N = 5$) for 20 peels were observed under 10 X and 40 X oculars. Stomatal frequency and trichome density were calculated by standard formulae (Salisbury, 1928).

Scanning Electron Microscopy (SEM)

For SEM, small strips (2.5 mm^2) of the leaf were trimmed along the middle portion of leaf blade from margin to midrib. The leaf segments were fixed in 5% glutaraldehyde and dehydrated in graded ethyl alcohol series. After dehydration and critical point drying, the leaf segments were mounted on metal stubs fitted in an ion sputter for gold coating (Fahn, 1986). Observations were made with JSM scanning electron microscope at University Science Instrumentation Centre (USIC), University of Delhi, New Delhi, India.

Data on SI, stomatal frequency and trichome density were analysed to obtain mean and standard error (SE) values for each treatment. The t-test was applied to assess the significance of differences between treatments and controls. .

Results

The content of Pb in soil influenced the growth of plants. Drastic reduction in plant biomass was observed at high levels (400 and 600 μg/g soil). Plants were short, showed sparse branching, and had chlorotic and necrotic leaves. The biomass and the plant height decreased by 50% and 42%, respectively in comparison to the untreated plants.

Stomata in cluster bean are anomocytic, anisocytic and amphicyclic (Fig. 1 A, B). Stomatal apertures on leaves of control plants were open, with smooth inner anticlinal walls (Fig. 1A, B), but at concentrations higher than 400 μg Pb, the apertures were partially or fully closed with thickened inner anticlinal walls (Fig. 1C). Structural changes were observed in stomata on the upper epidermis of plants treated with 400 or 800 μg/g Pb (Fig. 1C, D). Stomata were smaller and deformed in various ways. Abnormal stomata with single guard cells were observed in the leaves of plants treated with 600 or 800 μg/g Pb (Fig. 1D). Random

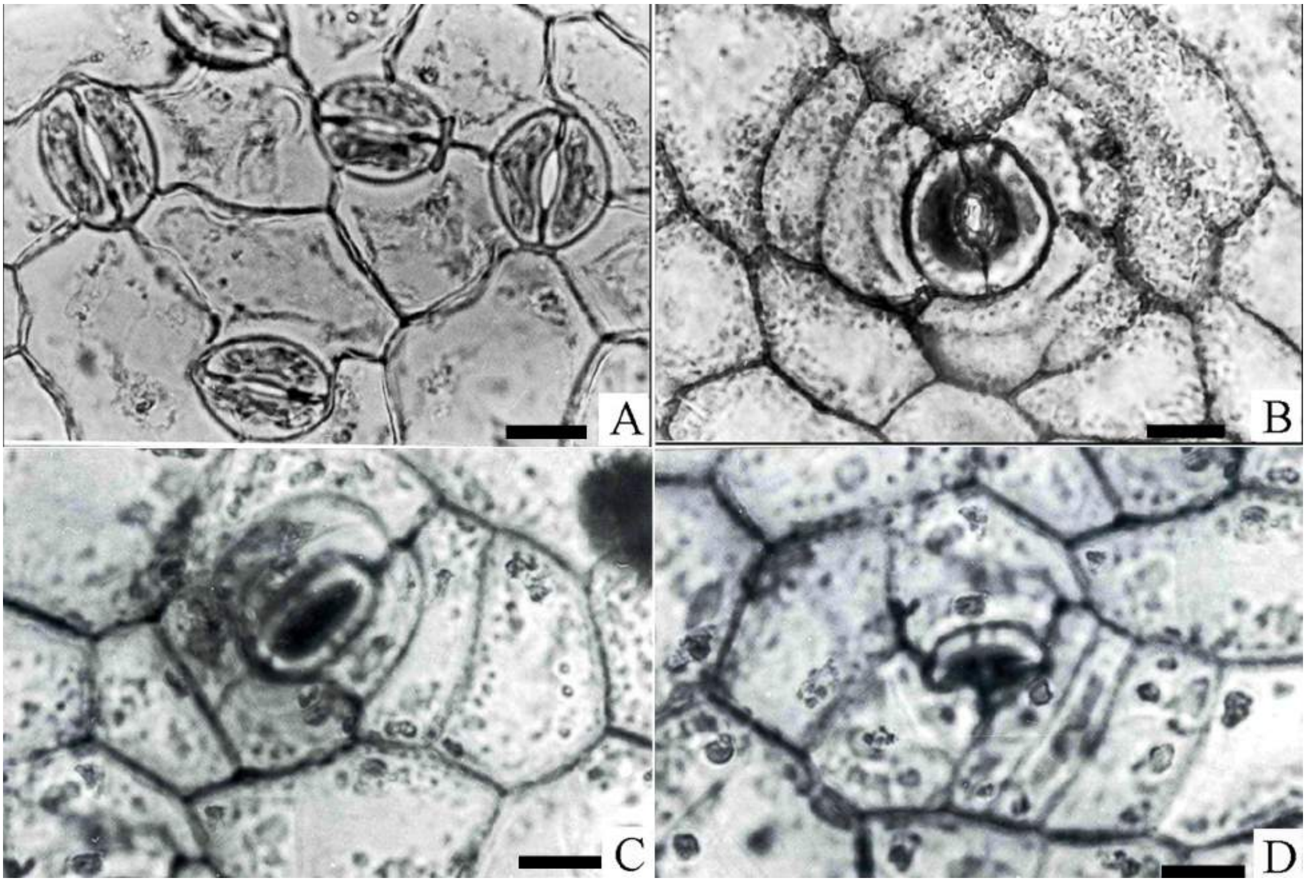


Fig. 1: Effect of lead treatment on stomatal structure seen in leaf peel mount. (A,B) Control showing normal anomocytic, anisocytic and amphicyclic stomata with well developed guard cells and large stomatal apertures. (A) Adaxial surface (B) Abaxial surface. (C,D) Lead treated plants showing abnormalities on adaxial surface. (C) Closed stoma with thickened inner anticlinal walls in leaf of a plant at 600 µg/g Pb. (D) Peel mount from adaxial surface of leaf epidermis of a plant at 800 µg/g Pb in soil showing stoma with one guard cell.

occurrence of contiguous stomata was also observed. There were a few abnormal stomata (2%) in the upper surface of leaves of untreated plants; in plants treated with 600 µg/g Pb, frequency of abnormality was high (20%) and at 800 µg/g it was as high as 43% (Fig. 2). Abnormal stomata were not seen in the abaxial (lower) epidermis of leaves of either untreated or treated plants.

The stomatal index (adaxial surface) was slightly higher at 100 and 200 µg/g Pb as compared with control, but was significantly higher (15-21%) at concentrations of 400-800 µg/g Pb (Table 1). On the abaxial epidermis, the effect was pronounced even at lower concentrations and was significantly higher (65-70%) at concentrations of 200-800 µg/g Pb. Stomatal frequency was higher at higher concentrations of Pb in the soil (Table 2). In leaves of control plants, stomatal frequency was 31.7% on the adaxial epidermis and 33.8% on the abaxial epidermis. Stomatal frequency was significantly higher on the adaxial epidermis at concentrations of Pb from 100 to 800 µg/g; stomatal frequency on the abaxial epidermis was significantly affected at concentrations of 400 µg/g and above (Table 2). The frequency of abnormal stomata rose with increasing concentrations of Pb (Fig. 2).

Trichomes, present on both surfaces of the leaf, were uniformly and densely distributed on both leaf surfaces of untreated plants but were sparse in leaves at concentrations of Pb of 600 µg and above (Fig. 3 A, B). This trend for lower trichome density at higher Pb concentrations was not statistically significant (Table 3).

Trichomes in leaves of control plants are glandular, T-shaped with a one to two-celled stalk and a single-celled head. The head has a uniform cuticle forming dome-shaped projections and covered by a thin layer

Table 1. Stomatal Index of adaxial and abaxial epidermis of leaves of plants grown at different concentrations of Pb in the soil, showing mean and standard error (S.E.) and results of t-test to test the statistical significance of differences between control and treatments.

Pb Concentration (μg/g of dry soil)	Upper Epidermis		Lower Epidermis	
	Mean±S.E.	*t*-value	Mean ±S.E.	*t*-value
Control	27.00±2.08	–	16.07±4.25	–
50	27.36±1.37	0.25$^{n.s.}$	19.94±2.59	0.96$^{n.s.}$
100	26.25±2.48	0.40$^{n.s.}$	21.57±1.56	0.67$^{n.s.}$
200	26.92±2.24	0.04$^{n.s.}$	23.95±1.79	2.96*
400	30.98±1.16	1.18*	24.12±1.96	3.20*
600	31.69±1.10	3.45*	26.53±2.28	2.65*
800	32.85±1.16	4.30**	27.39±1.59	5.21**

SI, stomatal index; n.s., non-significant; *significant at 5% level;**significant at 1% level.

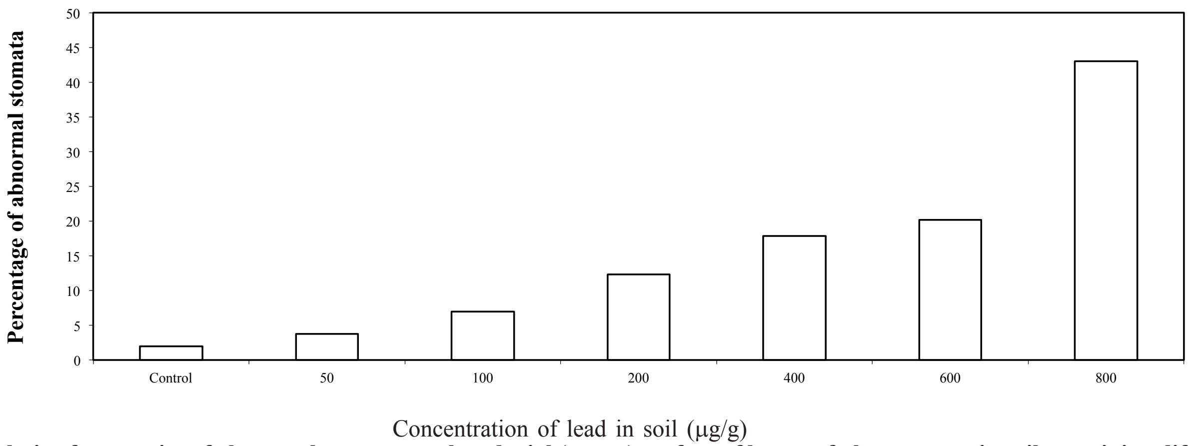


Fig. 2: Relative frequencies of abnormal stomata on the adaxial (upper) surface of leaves of plants grown in soil containing different concentrations of lead.

Table 2. Stomatal frequency on adaxial and abaxial epidermis from leaves of plants grown in soil with different concentrations of Pb, showing mean and standard error (S.E.) and results of t-test to test the statistical significance of differences between control and treatments.

Pb Concentration (μg/g of dry soil)	Upper Epidermis		Lower Epidermis	
	Mean ±S.E.	*t*-value	Mean ±S.E.	*t*-value
Control	31.73±3.08	–	33.88±4.50	–
50	35.24±2.23	1.60$^{n.s.}$	38.83±4.23	0.26$^{n.s.}$
100	28.22±2.09	3.03*	35.76±3.78	0.55$^{n.s.}$
200	41.57±2.14	4.55**	41.78±3.24	0.27$^{n.s.}$
400	43.20±1.63	5.70**	46.13±3.24	3.81**
600	47.45±1.42	8.06**	49.44±3.76	4.58**
800	48.05±0.82	8.91**	50.52±3.76	5.04**

n.s., non-significant; *significant at 5% level;**significant at 1% level.

of epicuticular wax (Fig. 3 A). However, in trichomes of leaves on plants grown in soil with 600-800 µg Pb, the cuticle and epicuticular wax layer were disrupted. The cuticle was seen to be discontinuous, and the wax was aggregated at some places. Wherever the cuticular covering was disrupted, pores in the cellulosic wall of the trichome head were visible (Fig. 2 C). Broken trichomes were also observed in plants at high Pb levels.

Discussion

The present study shows that high lead concentrations in soil had a significant impact on the leaf micromorphology. Stomata showed higher SI and frequencies, as well as abnormalities and permanent closure at higher levels of Pb in the soil. These responses are similar to those of plants exposed to air pollution -- higher SI, stomatal frequency and/or abnormal stomata in taxa such as *Amaranthus spinosus, Commelina benghalensis, Mangifera indica, Syzygium cumini* and other tree species, and several Fabaceae (Sharma et al., 1984; Vijayan & Bedi, 1986; Gostin, 2009 & Rai, 2016).

The walls around the stomatal aperture are far more thickened in stomata of leaves in lead-treated plants. The unusual thickening of the inner anticlinal walls of guard cells in treated plants seems to keep the stomata partially or completely closed, ostensibly to reduce water loss. Similar changes in the inner anticlinal walls of guard cells have been shown in response to Pb pollution (Ahmad et al., 2005). It has been suggested that permanently closed and abnormal stomata curtail gaseous exchange and reduce the impact of pollution stress Taylor(1978). However, because the present study reveals a response to Pb in the soil, this closure response is likely to be something other than a direct plastic response to water stress or reduction of gas exchange. Metal toxins such as arsenic and its metalloids have been found to interfere with polymerisation or depolymerisation of actin filaments and to alter the shape of guard cells (Hwang et al., 1997; Gupta & Bhatnagar, 2015). It is possible that lead has a similar impact on metabolic processes occurring during ontogeny of the leaf and differentiation of stomata, leading to abnormal stomata.

Normally stomata and trichome meristemoids are differentiated at specific distances from each other on account of their inhibitory influence or nutritional competition among them (Liakopoulos et al., 2006). Clustered trichomes in the leaves of Pb-treated plants in the present study suggest that such balance is disturbed. Trichome density may decrease at higher Pb concentrations on account of automobile exhaust in many plants because of irregular cell divisions. During the leaf development the cell division is altered and thus changes are set there in (Verma & Chandra, 2014).

Decrease in density and dimensions of trichomes in response to elevated lead levels seen in this study have also been observed in other plants, e.g., in other leguminous species such as *Vicia faba* and *V. radiata*,

Table 3. Trichome density on adaxial and abaxial epidermis from leaves of plants grown in soil with different concentrations of Pb, showing mean and standard error (S.E.) and results of t-test to test the statistical significance of differences between control and treatments.

Pb Concentration (µg/g of dry soil)	Upper Epidermis		Lower Epidermis	
	Mean ±S.E.	*t*-value	Mean ±S.E.	*t*-value
Control	7.22±0.09	–	10.69±2.07	–
50	7.20±0.09	$0.28^{n.s.}$	10.27±0.24	$0.35^{n.s.}$
100	7.17±0.05	$1.00^{n.s.}$	9.98±0.19	$0.59^{n.s.}$
200	7.06±0.05	3.28^{*}	9.80±0.39	$0.51^{n.s.}$
400	6.77±0.42	$1.87^{n.s.}$	9.66±3.10	$0.47^{n.s.}$
600	6.68±0.42	$2.25^{n.s.}$	8.84±0.43	2.32^{*}
800	6.31±0.40	$3.95^{n.s.}$	8.11±0.39	$1.51^{n.s.}$

n.s., non-significant; *significant at 5% level;**significant at 1% level.

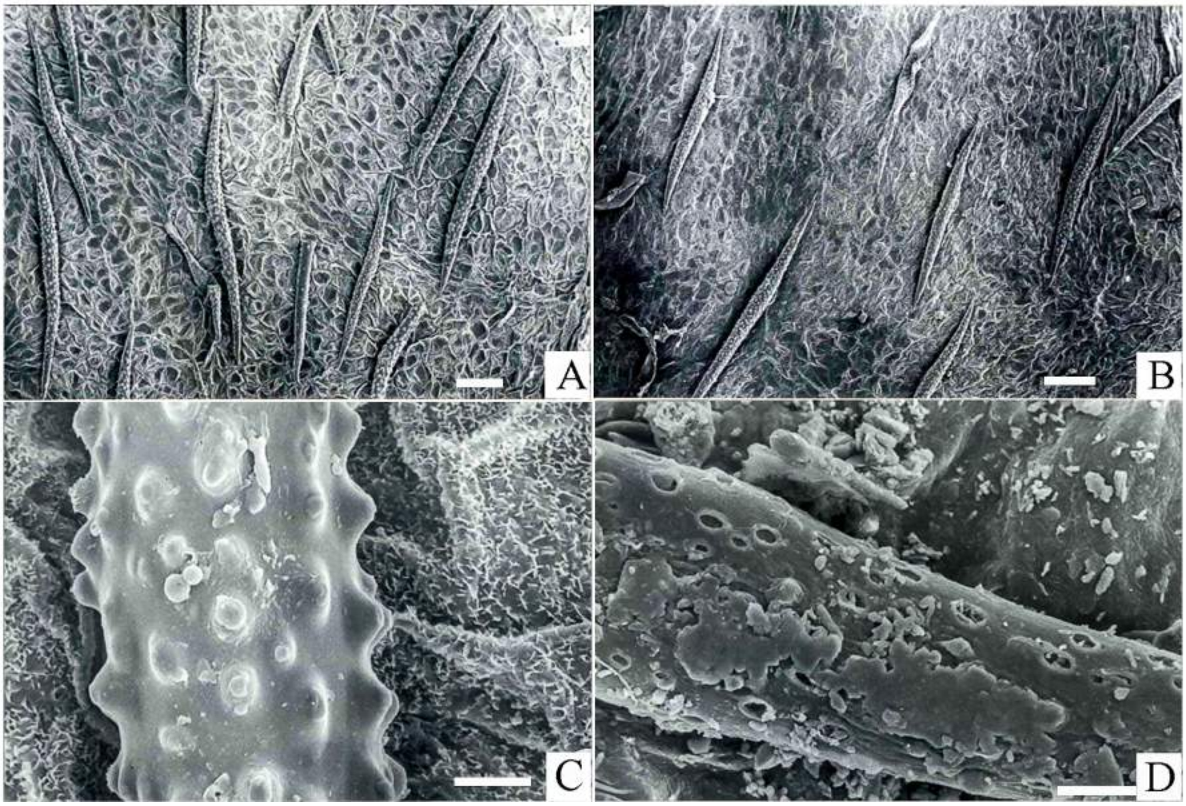


Fig. 3: Effect of lead treatment on trichome structure visualized by scanning electron micrography. (A, B) Differences in trichome density. (A) Control leaf showing normal high density; (B) Plant leaf at 600 µg/g Pb showing reduced density. (C, D) Cuticular projections and epicuticular wax on trichome surfaces. (C) Control leaf showing normal globular cuticular projections with a layer of epicuticular wax; (D) Plant leaf at 600 µg/g Pb in soil showing trichomes with disrupted cuticular cover and irregularly distributed epicuticular wax.

as well as in *Sida* and *Catharanthus,* where pollutants had a significant impact on density and distribution of trichomes and stomata (Neelu et al., 2000; Verma & Chandra, 2014; Hausser, 2014). Such reductions has been shown to be due to reduced resource availability or interference in vital processes such as photosynthesis, protein synthesis and the mitochondrial electron transport chain in other systems (Bittel et al., 1974; Barcelo et al.; 1988, Kastori et al., 1992; Willekens et al., 1997; Hauser, 2014). Our observations of disrupted cuticle on trichomes supports our viewpoint that the epidermal cells in treated plants have an impaired metabolic system because of which these are unable to develop normally. The trichomes seem to be functionless because normally the cuticular coverings help to retain the secretion of the glandular trichome in a sub-cuticular chamber so that it is released only on contact with the predators. Combined with decreases in density and length of trichome these changes could lead to decreased resistance toward insects under lead stress.

Acknowledgement

We thank UGC (University Grants Commission) for providing funds for undertaking this work.

References

Ahmad, S.H., Reshi, Z., Ahmad, J. & Iqbal, M. 2005. Morpho-anatomical responses of *Trigonella foenum graecum* Linn. to induced cadmium and lead stress Journal of Plant Biology, 48: 64-84.

Alves, E.S., Baêsso Moura, M. & Domingos, M. 2008. Structural analysis of *Tillandsia usneoides* L. exposed to air pollutants in São Paulo City–Brazil. Water Air Soil Pollution, 189: 61-68.

Barcelo, J., Vazquez, M.D. & Poschenrieder, C. 1988. Structural and ultrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris*). New Phytologist, 108: 37-49.

Bittel, J.E., Koeppe, D.E. & Miller, R.J. 1974. Absorption of heavy metal cations by corn mitochondria and the effects on electron transfer reaction. Physiologia Plantarum, 30: 266-280.

Chahal, V., Pair, C., Nagpal, A., Pakade, Y. & Katnoria, J. 2014. Evaluation of heavy metals contamination and its genotoxicity in agricultural soil of Amritsar, Punjab, India. International Journal of Research in Chemistry and Environment, 4: 20-28.

Duggal V., Rani, A. & Mehra, R. 2014. Monitoring of metal contaminations in groundwater in Northern Rajasthan, India. Journal of Environmental and Occupational Science, 3: 114-118.

El-Hassan, H.E., Diab, E.E.E. & Osman, G.A.M. 2014. Growth and metals accumulation in guar (*Cyamopsis tetragonoloba*) irrigated with treated wastewater. International Journal of Scientific and Technology Research, 3: 36-40.

Eun, S.O., Youn, H.S. & Lee, Y. 2000. Lead disturbs microtubule organization in the root meristem of *Zea mays*. Physiologia Plantarum, 110: 357-365.

Fahn, A. 1986. Structural and functional properties of trichomes of xeromorphic leaves. Annals of Botany, 57: 631-637.

Fragasaova, A 2001. Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapis alba* L. seedlings and their accumulation in roots and shoots. Biologia Plantarum, 44: 471-473.

Garg, K.K. & Varshney, C.K. 1980. Effect of air pollution on the leaf epidermis at the submicroscopic level. Experientia, 36: 1364-1366.

Gostin, I.N. 2009. Air pollution effects on the leaf structure of some Fabaceae species. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37: 57-63.

Gupta, P. & Bhatnagar, A.K . 2015. Spatial distribution of arsenic in different leaf tissues and its effect on structure and development of stomata and trichomes in mung bean, *Vigna radiata* (L.) Wilczek. Environmental and Experimental Botany, 109: 31-39.

Hauser, M-T. 2014. Molecular basis of natural variation and environmental control of trichome patterning. Frontiers in Plant Science, 5: 320.

Hwang, J.U., Su, S., Yi, H., Kim, J. & Lee, Y. 1997. Actin filaments modulate both stomatal opening and inward K^+ channel activities in guard cells of *Vicia faba* L. Plant Physiology, 115: 335-342.

Kaur, G., Kaur, R., Boparai, J.K., Sharma, V.K. & Sharma, P.K. 2017. Investigating the heavy metals and pesticides concentration in agricultural soil and groundwater of Mansa District of Punjab, India: insights into its impact on human health. International Conference on Recent Innovations in Engineering, Science, Humanities and Management (ICRIESHM-17) Panjab University.

Kalat, A. 2012. Soil and leaf lead concentrations in the Lincoln Park Area. DePaul Discoveries, 1: Article 8.

Kastori, K., Petrovic, M. & Petrovic, N. 1992. Effect of excess lead, cadmium, copper and zinc on water relations in sunflower. Journal of Plant Nutrition, 15: 2427-2439.

Krishna, A.K. & Govil, P.K 2004. Heavy Metal Contamination of Soil around Pali Industrial Area, Rajasthan, India. Environmental Geology, 47: 38-44.

Liakopoulos, G., Nikolopoulos, D., Klouvatos, A., Vekkos, K.A., Manetas, Y. & Karabourniotis, G. 2006. The photoprotective role of epidermal anthocyanins and surface pubescence in young leaves of grapevine (*Vitis vinifera*). Annals of Botany, 98: 257-265.

Manomita, P., Niladri, B., Bulbul, B. & Archana, S. 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environmental and Experimental Botany, 52: 199-223.

Mesmar, M.N. & Jaber, K. 1991. The toxic effect of lead on seed germination, growth, chlorophyll and protein contents of wheat and lens. Acta Biologia Hungarica, 42: 331-344.

Neelu, Tomar, M. & Bhatnagar, A.K. 2000. Influence of cadmium on growth and development of *Vicia faba* Linn. Indian Journal of Experimental Biology, 38: 819-823.

Olivares, E. 2003. The effect of lead on the phytochemistry of *Tithonia diversifolia* exposed to roadside automotive pollution or grown in pots of Pb-supplemented soil**.** Brazilian Journal of Plant Physiology**,** 15: 149-158.

Opeolu, O., Adenuga, O., Ndakidemi P. A. & Olujimi. O. 2010. Assessment of phyto-toxicity potential of lead on tomato (*Lycopersicon esculentum* L) planted on contaminated soils." International Journal of Physical Sciences 5, 2: 68-73.

Pourrut, B., Shahid, M., Dumat, C., Winterton, P. & Pinelli, E. 2011. Lead uptake, toxicity and detoxification in plants. Reviews of Environmental Contamination and Toxicology, 213: 113-136.

Press, M.C. 1999. The functional significance of leaf structure: A search for generalisations. New Phytologist, 143: 213-219.

Ram, H.Y.M. & Nayyar, V.L. 1974. A rapid method of obtaining epidermal peels in plants by treatment with cupric sulphate and hydrochloric acid. Stain Technology, 49: 114-116.

Rai, P.K. 2016. Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration. Journal of Asia Pacific, 9: 147-55.

Salisbury, E.J. 1928. On the causes and ecological significance of stomatal frequency with special reference to woodland flora. Philosophical Transactions of the Royal Society B: Biological Sciences, 216: 1-65.

Sengar, R.S., Gautam, M., Sengar, R.S., Garg, S.K., Sengar, K. & Chaudhary, R. 2008. Lead stress effects on physiobiochemical activities of higher plants. Reviews of Environmental Contamination and Toxicology, 196: 73-93.

Sharma, K., Sharma, A.P. & Agarwal, S.K. 1984. Effect of air pollution on epidermal characters of *Amaranthus spinosus* L. and *Commelina benghalensis* L. Acta Ecologia, 6: 1-3.

Taylor, G.E. 1978. Plant and leaf resistance to gaseous air pollution stress. New Phytologist, 80: 523-534.

Tian, D., Tooker, J., Peiffer, M., Chung, S.H. & Felton, G.W. 2012. Role of trichomes in defense against herbivores: comparison of herbivore response to woolly and hairless trichome mutants in tomato (*Solanum lycopersicum*). Planta, 236: 1053-1066.

Verma V. & Chandra N. 2014. Biochemical and Ultrastructural Changes in *Sida cordifolia* L. and *Catharanthus roseus* L. to Auto Pollution. International Scholarly Research Notices 1-11.

Vijayan, R. & Bedi, S.J. 1986. Use of leaf epidermis in air pollution impact study, In Proceedings of National Seminar on Environment Pollution Control and Monitoring. 274-278 pp. Central Scientific Instruments Organization, Council of Scientific and Industrial Research, Chandigarh, India.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inzé, D. & Van Camp, W**.** 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defense in C_3 plants. EMBO J., 16: 4806-4816.

Everything natural is not safe

By **Daily Excelsior** - 25/11/2018

Monika Koul

With foggy mornings and chilly evenings at your doorstep, weather and associated illnesses are increasing rampantly. Respiratory conditions such as upper and lower respiratory tract infections (common cold, flu and pneumonia) occur commonly, while asthma and chronic bronchitis worsen during the cold season. Lack of bright sunlight pushes sedentariness that further leads to lethargy and increase in the fat levels in the body along with low Vitamin D content that affects the bones and joints. Doctors and medical practitioners are busy and hospitals overcrowded. Many people look for alternative therapies for cure of chronic ailments and fortunately, therapeutic herbal treatments, medicinal plant herbs, fruits, roots, rhizomes, corms of various wild plants are used by a vast chunk of population. Besides, herbal drugs are also patronised, advertised more these days because of which we can see many of these products on pharma shelves across the board. Many plants, their products and decoctions made from these have a profound influence on respiratory afflictions. Demulcent herbs contain mucilage which soothes dry, irritated tissues; they are specific treatment for a sore throat or a dry cough. Antitussive agents reduce respiratory spasms and are most beneficial for coughs which have a dry, irritated character and for the treatment and maintenance of asthmatic conditions. Many plants have analgesic, antiseptic and anti-inflammatory properties and hence are used in ointments, syrups and massage oils. However, despite their continuous use in various forms as ingredients of some health boosting stuff available in market, some other major health issues have started cropping up in the users. If one issue is getting addressed, other issues also need immediate attention.

There is a general belief amongst the consumers globally that herbal drugs are always safe because they are “natural”. Herbal drugs and medicines made from these are being overestimated for their efficacy. People have started consuming these in quantities that are undefined and dosage is considered insignificant thing for these. Herbal medicines are managed as food supplements, functional food, health products, or drugs with differential standards and chaotic market.

A recent study conducted by us at Hansraj College, under the Major Research Project funded by University Grants Commission, Government of India looked at some of the common medicinal plants that are used to treat various ailments in North India. We collected Solanum nigrum (makau), Withania somnifera (Ashwgandha) and Adathoda vasica (Vasa/Vasaka) from various places in and around Delhi and found that the plants are eaten as vegetables, used as massage ingredients, applied externally and there are plenty of herbal drug formulations available from various noted brand names. The study revealed that plants grown especially, in nurseries are irrigated with polluted water. The leaves, stems, roots and fruits were subjected to some analytical procedures, the Atomic Absorption Spectrometry (AAS) and biochemical analysis. We deciphered that all the three medicinal plants showed the presence of heavy metals such as Pb (Lead), Cadmium (Cd), Chromium (Cr) and Nickel (Ni).

The plants that were growing in wild had metals much below the permissible limits but those growing in cultivation have high levels that can get absorbed in the body tissues and concentrate there in. The concern is that these heavy metals are going through medicines and have long term health implications that are going unnoticed. Cd, Pb, Ni, Cr are toxic to human health and cause various problems related to bones, kidneys and even dementia in adults. There have been incidents when the herbal medicine consignments from India have been tested in United States for the presence of heavy metals and have been out rightly rejected. However, here in our own country and in many states of North India, use of medicinal plants is in the form of self -medication and local practitioners. The people prescribing the medicines are not supposed to have a mandatory license and most important our claim of grandmas prescription is well justified. Our studies clearly indicate that if the collection of the plants is done from sites where soil is polluted or plants are irrigated with polluted water a cautious approach is required. Plants such as Makau, Ashwgandha and Vasaka should not be randomly prescribed, as these are freely available. The plants if contaminated can be very harmful to pregnant women, the unborn foetus and young children. Besides there is limited data on the efficacy and safety of their formulations on various body ailments hence should not be over-consumed. A regulatory framework for herbal medicines should be set up and all vendors that sell these should come under surveillance to ensure the quality and safety of herbal medicines is maintained. Otherwise, a remedy can be a disaster in the long run. So watch out, ensure the medicine does not turn into a toxin.

(The author is Assistant Professor Department of Botany Hans Raj College, University of Delhi)
feedbackexcelsior@gmail.com

National Conference on

Plant Science Research : Looking Beyond 21^{st} Century for Environmental & Agricultural Revolution

PROGRAMME & ABSTRACT

Feb 5^{th} - 7^{th} 2016

Organized by :

Society for Plant Research (VEGETOS)

www.vegetosindia.com

&

Department of Botany

University of Delhi, Delhi – 110 007

Society For Plant Research ★ Estd. 1988 ★ INDIA

UNIVERSITY OF DELHI

In vitro effect of dual stress of salt and lead on the growth parameters of *Eucalyptus camaldulensis* seedlings

P-105

Roma Rani* and Suresh Kumar

Ramjas College, University of Delhi-110007

*Email: ganga606@gmail.com

Considering the seriousness of salinity and heavy metal pollution (Pb) a set of experiments was conducted under *in vitro* controlled conditions using 100 mM salt (NaCl) with 25-200 mg/l lead (PbAc) and without lead (control) in *Eucalyptus camaldulensis* seeds. A combinational stress (lead + salt) proved to be less harmful instead of individual stress (salt), for growth indices. *E. camaldulensis*, well known for its moderate salt tolerance ability. Presence of lead (PbAc) in the MS medium supplemented with 100 mM salt (NaCl) led to increment in the % seed germination, seedling length and stress tolerance index compared to the control (lead free). Maximum shoot length and % seed germination was recorded at 100 mg/l PbAc with 100 mM NaCl, while maximum root length and % tolerance index was achieved at highest amount of lead (200 mg/l) with the combination of salt (100 mM) compared to the control (lead free, containing 100 mM NaCl). Performance of seedlings in the presence of salt and lead indicate the possibility of exploring the potential of *E. camaldulensis* saplings in such contaminated sites.

Heavy metal uptake and contamination in medicinal plants: Hype or reality

P-106

Nikita Sharma* and Monika Koul[1]

Department of Botany, University of Delhi, Delhi - 110 007

[1]*Hansraj college, Department of Botany, University of Delhi, Delhi - 110 007*

*Email: nikita450124@gmail.com

Medicinal plants have been widely used since time immemorial to cure various ailments. The World health organisation (WHO) estimates that 4 billion people or 80% of the world population, presently use herbal medicine for some aspect of primary healthcare and 25% of modern medicines are made from plants first used traditionally. Resistance to allelopathic drugs used for primary healthcare is cause of major concern throughout the world. Besides that many of the allelopathic drugs also have proven side effects. Therefore, use of medicinal plants and their end products is increasing. However, most of the medicinal plants are growing in wild and in wetlands. These plants are collected from various places and no special methods are used by practioners to test the safety aspects. *Solanumnigrum*, *Withania somnifera*, *Plumbago zeylanica*, and many more are used for treatment of various ailments and their parts such as roots, leaves and fruits are also used ethanobotanically by the local people. There are reports that some of these plants are metal accumulators. As the pollution level is increasing all across the habitat on account of anthropogenic activities, it is important to assess the uptake of heavy metals in the plants and their parts. This study will help in assessing the safety as well as efficacy of the drugs derived from the plants.

Medicinal Plant Research 2015, Vol.5, No. 4, 1-9
http://mpr.biopublisher.ca

Research Article Open Access

Effect of arsenic on trichome ultrastructure, essential oil yield and quality of *Ocimum basilicum* L.

Biswas S.[1], Koul M.[2], Bhatnagar A.K[1]
1.Department of Botany, University of Delhi, Delhi – 110 007, India
2.Department of Botany, Hans Raj College, University of Delhi, Delhi – 110 007, India
Corresponding authors email: drmkoul@gmail.com
Medicinal Plant Research, 2015, Vol.5, No.4 doi: 10.5376/mpr.2015.05.0004
Received: 07 Jun., 2015
Accepted: 15 Jul., 2015
Published: 07 Aug., 2015

Preferred citation for this article:
Biswas S., Koul M., Bhatnagar A.K., 2015, Effect of arsenic on trichome ultrastructure, essential oil yield and quality of *Ocimum basilicum* L., Medicinal Plant Research, 5(4): 1−4 (doi: 10.5376/mpr.2015.05.0004)

Abstract An experiment was carried out to study the effect of various arsenic (As) treatments on growth, essential oil (EO) yield, composition of oil and morphology and ultrastructure of glandular trichomes of *Ocimum basilicum* (sweet basil) an important EO yielding plant. As in the form of disodium hydrogen arsenate [Na2HAsO4.7H2O] was added in the soil in the range of 0, 10, 50, 150 mg/kg As. As stress caused reduction in growth and biomass of shoot system at 50 and 150 mg/kg As. EO yield increased by 3.5-4 times at 10 and 50 mg/kg As, but decreased significantly by 0.08% at 150 mg/kg As. GC analysis revealed that linalool the main EO compound present in the leaves augmented 3 to 4 times under 10-150 mg/kg As as compared to control. Other compounds such as 1,8-cineol and methyl eugenol decreased with increased As treatments whereas methyl cinnamate was not detected at 50 and 150 mg/kg As in gas chromatographs. Camphor did not appear in any of the As treated plants. Light microscopic studies and electron micrographs revealed that As stress affected glandular trichome morphology and ultrastructure. Premature senescence was observed in trichomes of leaves at 150 mg/kg As and noticeable changes were observed in cell organelles of secretary cells. A positive correlation between EO yield and trichome density was observed in the present study.
Keywords Arsenic, Essential oils, *Ocimum basilicum,* trichomes

1. Background

Contamination of soil with As and As-containing salts is a global environmental problem. As-based pesticides, fertilizers, metal processing industries and coal combustion units are some of the main sources of As pollution (Meharg and Whitaker, 2002; Liao et al., 2004). Pollution of groundwater with As is increasing at an unprecedented rate in some South Asian countries, especially Bangladesh and India (Ghosh et al., 2006). Irrigation of agricultural fields with As polluted water has significantly increased As levels in soil (Marin et al., 1992). Consumption of food grown in As-contaminated soil causes many health problems such as cancer, cardio-vascular disease and neurological disorders (Gadepalle et al., 2008). As polluted water has the potential to cause severe skin allergies, dermatological lesions and other health related problems.

Persistence of As in the environment for long duration is resulting in decrease in yield and quality of many important agricultural crops (Rashid et al., 2004). As is taken up from the soil by plant roots and is transferred to higher trophic levels via food chain (Zhang et al., 2002). Experiments carried out on a variety of crop species have demonstrated that As contamination in soil causes adverse effects on plant biomass productivity and yield (Carbonell-Barrachina et al., 1997). As uptake by plants and its effect on plant nutrition has been investigated in detail for various species such as *Brassica juncea*, *Oryza sativa*, *Pteris vittata* and *Spartina alterniflora* (Carbonell et al., 1998; Abedin and Meharg, 2002; Chaturvedi, 2006; Fayiga et al., 2007).

EO yielding plants are important cash crops and are sources of bioactive constituents that have medicinal value. These plants are considered to be hardy and grow safely on metal-polluted soils around smelters and soil contaminated with heavy metals (Zheljakov and Nielsen, 1996; Salamon, 2008). Studies conducted on some important medicinal plants such as *Bidens tripartite*, *Leonurus cordiaca*, *Marrubium vulgare*, *Melissa officinalis* and *Origanum heracleoticum* clearly depict that no severe phytotoxic symptoms were observed in morphology, EO percentage and yield of these plants (Zheljakov et al., 2008). Similarly, EO yield of *Mentha piperita* and *Ocimum basilicum* is also not affected by the treatments of Cd, Pb and Cu in soil. However, application of these heavy metals can alter the composition of EO (Zheljakov et al., 2006).

Ocimum basilicum L. (Lamiaceae), commonly known as sweet basil is an annual aromatic medicinal herb native to India and other regions of Asia. In India, basil is mainly cultivated in Assam, Bihar, Uttar Pradesh and West Bengal where soil is severely polluted with As (Heikens, 2006; Rao et al., 2007). It grows luxuriantly in variety of soil types and agroclimatic conditions (Begum et al., 2002). EO derived from basil is widely utilized in high-grade perfumes, aromatherapy, flavoring liquors and as herbal spice (Bahl et al., 2000; Kumar et al., 2004). EO contains biologically active constituents that possess antimicrobial (Elgayyar et al., 2001), fungistatic (Reuveni et al., 1984), insecticidal (Bowers and Nishida, 1980) and allelopathic properties (Rice, 1979). Traditionally, basil has been used for the treatment of headache, cough, diarrhoea, constipation, warts and kidney malfunction (Politeo et al., 2007). The yield and composition of EO is dependent on many environmental factors, agrochemical practices and the type of cultivar (Jirovetz et al., 2003).

Leaves of *O. basilicum* bear non-glandular and glandular trichomes on both the abaxial and adaxial surfaces. Non-glandular trichomes are uniseriate, pointed, straight or hook-like and their function is to confer defence to plants. Glandular trichomes are responsible for biosynthesis, secretion and accumulation of EO (Fischer et al., 2011). According to Wolff et al. (2012) high concentration of heavy metal in soil affects the structural integrity of the trichomes. However, there is no detailed experimental evidence to address how trichomes respond to high As levels in soil, and how the alteration of trichome structure influences the EO yield and quality. Studies on how ultrastructure of glandular trichomes is affected in treated plants and how cell organelles of functional importance such as endoplasmic reticulum (ER), mitochondria and plastids respond to high As concentrations has not been carried out in relation to As toxicity.

The present study was designed to investigate: (i) effect of different As concentrations in soil on EO yield and composition of basil plants; (ii) impact of As in the soil on morphology and ultrastructure of EO secreting glandular trichomes. The study has been undertaken to understand the EO secretion and glandular trichome function in uncontaminated (control) and As- contaminated soil.

2. Results

1.1. Effect of As on growth

Table 1 shows that *O. basilicum* plants exposed to increasing As concentrations showed progressive decrease in the vegetative growth i.e. shoot length and shoot biomass in terms fresh and dry weight. Highest reduction was observed at 150 mg/kg As. Shoot length was significantly reduced by 34.65%, compared to control, while difference between other treatments was not significant. Shoot fresh and dry mass accumulation was inhibited by 31.78 and 27% respectively at 150 mg/kg As. However, an increase in shoot length, fresh and dry weight was observed at 10 mg/kg As.

1.2. Effect of As on EO yield and composition

Hydrodistillation of leaf extract from the three month old basil plants was carried out. EO yield of the control plants was 0.20% (w/w). At 10 and 50 mg/kg As, EO yield significantly increased 3.5-4 times to reach 0.77 and 0.8% respectively, whereas at 150 mg/kg As the yield significantly reduced up to 0.08% (Figure. 1). EO components estimated in control plants were linalool (0.0013 mg/g DW), methyl cinnamate (0.013 mg/g DW), camphor (0.0072 mg/g DW), 1,8-cineol (0.009 mg/g DW) and methyl eugenol (0.028 mg/g DW). Linalool concentration increased 3-4 times under 10-150 mg/kg As as compared to the control. Methyl cinnamate concentration at 10 mg/kg As showed down regulation up to 72.30%, whereas at 50 and 150 mg/kg As it was not detected. Concentration of 1,8-cineol and methyl eugenol decreased by 67.7 and 96% respectively at 150 mg/kg As in comparison to EO extracted from leaves of control plants. Camphor was severely affected by As toxicity and not detected at all in any of the As-treated plants (Table 2).

1.3. Effect of As on trichome density and development

1.3.1. Trichome density

On the adaxial surface, trichome density increased 2-3 times at 10 and 50 mg/kg As in comparison to control plants. At 150 mg/kg As, trichome density decreased by 34%. Trichome density on abaxial epidermis increased at 10 mg/kg As by 66%, while at 50 and 150 mg/kg As it decreased by 67 and 34 % respectively as compared to control (Figure. 2).

1.3.2. Trichome *structure and ultrastructure*

LM studies showed that glandular trichomes on the leaves of control plants were globular, with a stalk of 1-3 cells and a head of four secretory cells (Figure. 3A). At 10 and 50 mg/kg As in soil structural collapse of the trichome head by disorganization of the secretory cells and folding of their cell walls was observed. Trichomes of plants treated with 150 mg/kg As showed senescence-like symptoms. Head cells showed mushroom-like appearance, partially protruding out of the epidermal depression (Figure. 3D). SEM investigations revealed an equatorial line of weakness around the head of the trichome in leaves of control plants (Figure. 4A). The cuticle ruptured along this line, and the subsequent collapse of the subcuticular cavity led to the release of exudates. Early maturity of trichomes was observed in As treated plants. Disintegration of the secretory cells and folding of the cell walls was observed in trichomes at 10, 50 and 150 mg/kg As treatments. Exposed head cells with no cuticle were noticed at 50 mg/kg As (Figure. 4C). A large number of deeply grooved trichomes was common at 150 mg/kg As (4D), a typical behaviour of mature trichomes (Werker, 1993). Wax deposition was more clearly visible on leaf surface (Figure. 4D). Distortion of epidermal cells was observed with increased concentration of As. Non-glandular trichomes also lost the structural integrity with higher doses of As in soil.

TEM studies provided further insights into the secretory cells of trichomes. Well-developed central nucleus and dense cytoplasm was observed in the head cells of control plants. Large vacuoles and mitochondria having well developed cristae were seen all over (Figure. 4E). Rough endoplasmic reticulum (RER) and dictyosomes were also distinctly visible, signifying that cells were actively involved in EO secretion. At 10 mg/kg As no significant variation was observed in head cells in comparison to control. Well developed nucleus with electron dense chromatin material was observed in the cells (Figure.4F). However, at 50 mg/kg As, plasmodesmatal connections were

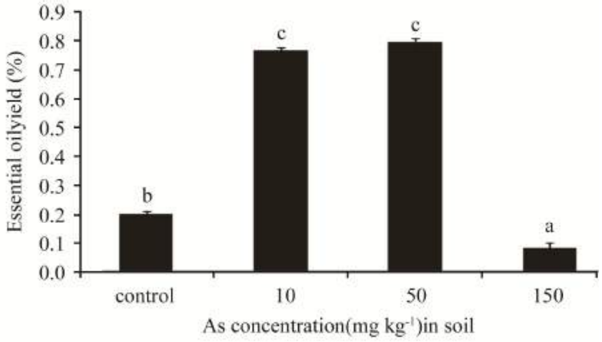


Figure 1. EO yield (%) of *O. basilicum* as affected by different concentrations of arsenic levels. Values with different superscripts (a-c) are significantly different at $p \leq 0.05$.

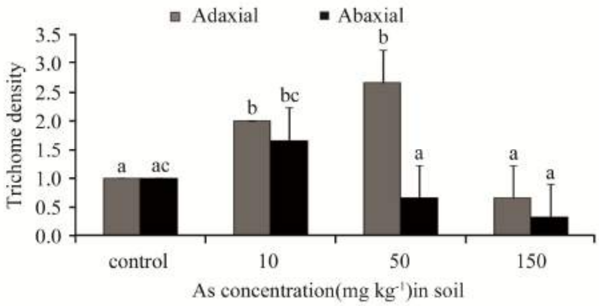


Figure 2. Trichome density (cm^2) on both adaxial and abaxial surfaces of *O. basilicum* as affected my different arsenic levels. Values with different superscripts (a-c) are significantly different at $p \leq 0.05$.

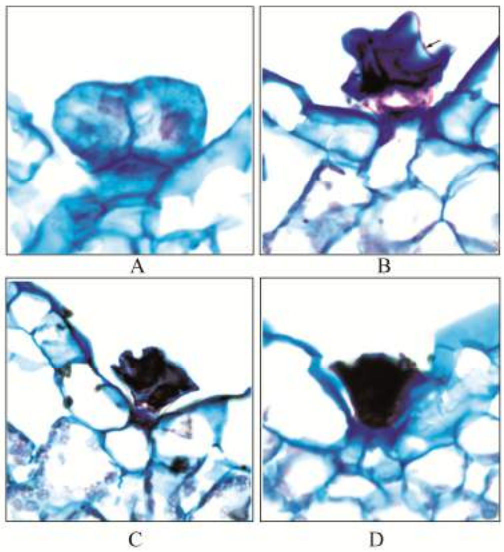


Figure 3. Light micrographs showing the response of O. basilicum leaf peltate glandular trichomes to arsenic at different concentrations (40X).

(A)Control: Developing trichome.

(B)10 mg/kg As: Folding of head cells (arrow).

(C)At 50 mg/kg As: Collapsed head cells and shortening of stalk cell.

(D)At 150 mg/kg As: Senescent trichome.

Table 1 Effect of arsenic on shoot length, fresh wt. and dry wt. of Ocimum basilicum.

Arsenic (mg/kg soil)	Shoot length	Shoot FW	Shoot DW
0	47.33±1.16 c	36.50±2.50 c	3.10±0.17 b
10	68.96±0.87 d	60.33±2.08 d	5.16±0.37 c
50	40.90±1.65 b	32.73±1.55 b	3.23±0.61 b
150	30.93±1.44 a	24.90±0.65 a	2.26±00 a

Values with different letters (a-d) are significantly different at $p \leq 0.05$ (means of three replicates ± S.D.).

Table 2 Effect of arsenic on essential oil composition of *Ocimum basilicum*.

Compounds	0 mg/kg	10 mg/kg As	50 mg/kg As	150 mg/kg As
Methyl cinnamate	0.013±0.005	0.0036±0.00	nd	nd
Linalool	0.0013±0.00 a	0.005±0.00 c	0.0047±0.00 bc	0.004±0.00 b
Methyl eugenol	0.028±0.00 e	0.006±00 bd	0.008±0.00 cd	0.001±0.00 a
1,8-cineol	0.009±0.001 c	0.005±0.00 b	0.0036±0.00 a	0.0029±0.00 a
Camphor	0.0072±0.00	nd	nd	nd

nd: not detected Values with letters (a-e) are significantly difference at $p \leq 0.05$ (means of three replicates ± S.D.).

more prominent on the secretory cell wall and large mitochondria were seen (Figure 4. 4G). At 150 mg/kg As, glandular trichomes showed various changes at structural and ultrastructural level. The sectretory cells showed less number of vacuoles. RER were well represented but size of mitochondria was observed to be reduced (Figure. 4H).

2. Discussion

Results of the present study clearly demonstrate that the growth of *O. basilicum* significantly decreased as a result of higher As phytotoxicity, though at lower As concentrations the effect was not significant. An increase in plant growth at 10 mg/kg As in soil was observed. Results are in agreement with the study on *Scutellaria biacalensis*, an important herbal plant used in traditional Chinese medicine where low levels of As in soil stimulated the growth and development (Cao et al., 2009). This positive response at lower As concentrations can be linked to phosphorous uptake. Phosphate and arsenate are taken into plant roots by a common carrier. However, the phosphate/plasma membrane carrier has much higher affinity for phosphate than arsenate (Meharg and Macnair, 1990). Phosphate is also reported to be an efficient competitive inhibitor of arsenate uptake (Meharg and Macnair, 1990). At low soil As concentration, displacement of soil phosphate by arsenate increases the availability of phosphate to the plants, which results in the increase of growth.

Four-fold increase in EO yield in plant tissues was observed when As concentration in the soil was raised up to 50 mg/kg, whereas at 150 mg/kg As the oil yield significantly decreased by about 60% in comparison to control. Heavy metal-induced enhancement in EO yield at low concentration in growth medium has been reported earlier in other EO yielding species such as *Matrica chamomilla* (Nasiri et al., 2010), *Mentha piperita* (Prasad et al., 2010) and *Salvia officinalis* (Stancheva et al., 2009). Increase in the EO content by the application of heavy metals is not properly understood, but the change has been attributed to the effect of metallic elements on the enzyme activity and carbon metabolism which further affects the EO synthesis pathways (Prakash and Kardage, 1980). Increase in EO production and density of EO releasing trichomes in *O. basilicum* has been observed in the present study at moderate As levels. This can be a partial explanation for the observed higher oil content per unit leaf dry weight. According to Charles et al. (1990) EO accumulation increases indirectly by interfering with net assimilation rate of nutrients in plants or by unequal partitioning and distribution of resources for growth and differentiation. EO production in basil leaves was strongly inhibited at 150 mg/kg As. Reduction in photosynthesis and/or additional changes in metabolic system are probably responsible for this inhibition. According to Croteau and Johnson (1984) EO biosynthesis takes place in epidermal oil glands that are carbon heterotrophic and thus depend on the adjoining photosynthesizing cells

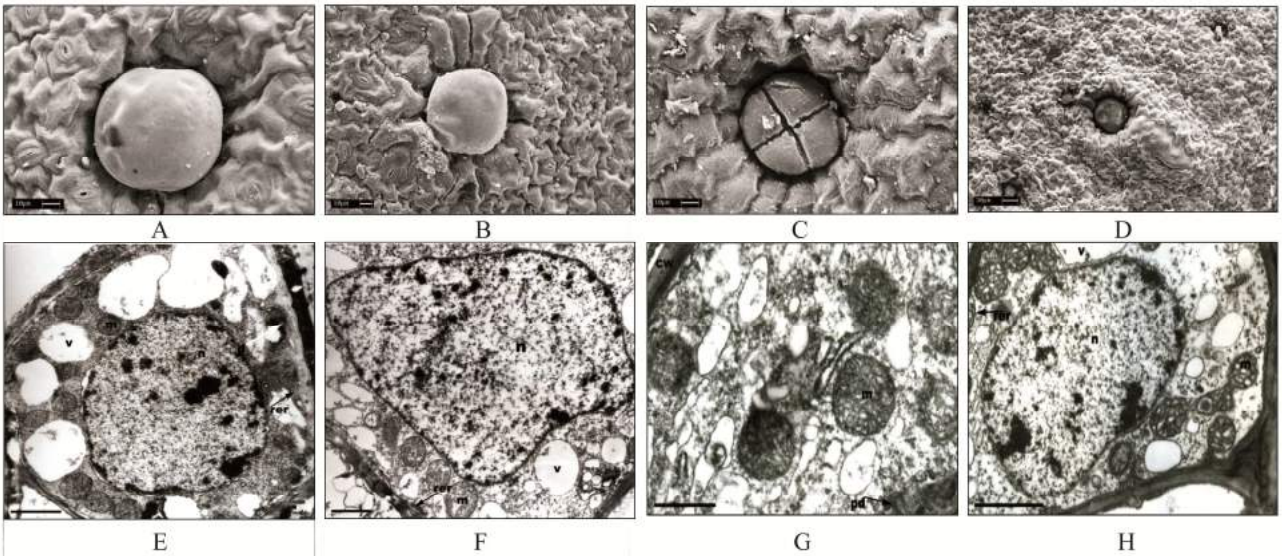


Figure 4 Scanning electron micrographs of O. basilicum showing effects of different concentrations of arsenic on morphology of peltate glandular trichomes.

(A) Control: Mature peltate trichome. Arrow indicates cuticle in central part showing a buldge, possibly corresponding to sub-cuticular cavity underneath.

(B) 10 mg/kg As: Mature peltate trichome on adaxial leaf surface.

(C) 50 mg/kg As: Mature peltate trichome with torn cuticle sheath, disclosing the head cells.

(D) 150 mg/kg As: Sunken trichome.

Transmission electron micrographs of glandular cells of peltate trichome in the active secretory stage under different concentrations of arsenic. (cw: cell wall, rer: rough endoplasmic reticulum, m: mitochondria, n: nucleus, v: vacuole, d: dictyosome, pd: plasodesmata). (Bar = 1μm)

(E-F) Control and 10 mg/kg As: Details of cytoplasm and organelles in secretory cells.

(G) 50 mg/kg As: Large-shaped mitochondria. Plasmodesmata in the secretory cell walls (arrow).

(H) 150 mg/kg As: Secretory cell showing peripheral nucleus and mitochondrial aberrations in the form of inflated cristae.

for a continuous supply of carbon precursors. EO production is also dependent on availability of various nutrient ions in soil. Any disruption in the nutrient balance reduces the oil production as observed in basil plants exposed to higher level of As in soil. Such results have also been observed in plants growing in saline soils. Reduction in EO yield due to high dose of 100 mM NaCl has been observed in *Salvia officinalis* (Ben Taarit et al., 2009) and *Origanum majorana* (Baatour et al., 2010). As in soil disturbs the ionic balance and availability of nutrient ions to plant systems as observed in response to salinity. Comparison of plant biomass and oil content at different treatments revealed that As stress showed more severe effect on biomass than on oil content. This low reduction of oil content is certainly an advantage for EO plants. Biswas et al. (2011) in a detailed review have attributed the changes in oil yield in various EO plants to cascading effects of stress imposed on account on salt, heavy metal or water availability. Stress encountered by the plants affects the presence and availability of nutrient elements, and hence the secretory pathway.

Ultrastructual studies of secretory structures of trichomes showed early maturity in plants grown in As amended soil. At 150 mg/kg As, structure of head cell changed from globular to mushroom-like and the trichomes were seen embedded into the epidermal surface. Such changes are generally observed in trichomes at post secretory stage (Gravano et al., 1998). Werker et al. (1993) also observed that in *O. basilicum* glandular trichomes were embedded in the epidermis when trichomes attain maturity and proceed to senescence. Premature senescence of trichomes is distinctly observed in the present studies in response to As toxicity.

At 10 mg/kg As trichome ultrastructure did not show any variation in comparison to trichomes of control plants. At both the treatments, the head cells showed highly-organized cytoplasm, large nuclei, and many mitochondria. These features are typical of secretory tissues with high metabolic activity. Secretion is an active process in which energy is used for metabolic compartmentation, ion extrusion, or biosynthesis of products (Fahn, 1988). Hence, presence of a large number of mitochondria in glandular trichomes is indicative of active secretion. Presence of large vacuoles is related to the storage of metabolites and ions in the secretory apparatus (Figueiredo and Pais, 1994). At 50 mg/kg As, trichomes of *O. basilicum* showed extensive RER and large mitochondria, indicating that the activity was higher in glandular cells that stimulated the production of EO. The existence of more prominent plasmodesmata connecting the cytoplasm of the secretory cells indicates enhanced intercellular transport of compounds within the trichome. Mitochondrial aberration in the form of inflated cristae and less electron dense cytoplasm was seen at 150 mg/kg As. Underdeveloped organelles in the trichomes at 150 mg/kg As could be the possible reason for low yield in EO. This clearly demonstrates that higher doses of As affect the cells and disrupt their metabolic machinery. The variation observed in yield are manifestations of changes observed at cellular and subcellular levels.

Characteristic basil aroma appears due to 1,8-cineol, methyl cinnamate and linalool (Lee et al., 2005). Camphor, 1,8-cineol and linalool are also known to be biologically active components (Morris et al., 1979). These compounds possess antimicrobial and antioxidative properties with high therapeutic value (Raseetha et al., 2009). GC analysis revealed that linalool, the major constituent in *O. basilicum* was not affected by exposure to As, but relative concentration of methyl eugenol, methyl cinnamate, 1,8-cineol and camphor diminished when plants were subjected to As stress (Table 2). Such a change in constituents of EO can be considered detrimental to oil quality. Low concentration of 1,8-cineol and absence of camphor at higher As levels depict that there is a compromise in defense potential as these two constituents are involved in allelopathic reactions (Rice, 1979). Compounds responsible for typical aroma of basil are affected due to As toxicity, which also degrades the antioxidative property of basil. Such changes in the EO composition in response to heavy metal stress have also been reported in *Rosmarinus officinalis* (Deef, 2007), *Mentha arvensis* (Prasad et al., 2010) and *Salvia officinalis* (Stancheva et al., 2009). Compromise on quality of therapeutically important compounds reflect inactivation of enzymes or redirection of metabolic functions to maintain growth (Murch et al., 2003).

3. Material and Methods

3.1. Plant material, growth conditions and treatments

Seeds of *Ocimum basilicum* were procured from Germplasm Conservation Division, National Bureau of Plant Genetic Resources (NBPGR), New Delhi (India). Pot culture experiments were conducted in Botanical Garden, University of Delhi, Delhi (India). Seeds were sown in October in pots (38 cm diameter) filled with 4 kg air dried soil and compost in equal ratio. Soil was clay loam, pH 7.2, NO_3 N 125 mg/kg, available P 0.5 mg/kg and K 120 mg/kg. Air-dried soil was amended with disodium hydrogen arsenate [$Na_2HAsO_4.7H_2O$] at concentrations of 10, 50 and 150 As mg/kg soil, each with three replicas. Pots without As served as control. Twenty seeds were sown in each pot and five uniformly growing seedlings per pot were retained for further studies. Plants were grown under natural conditions of light, temperature and humidity.

Plants were harvested after four months of growth period. Whole plants were scooped out. Fresh weight of the shoot system was taken. Plant tissue was oven dried at 72 ^{o} C and weight was recorded.

3.2. EO extraction

Fifty grams of dried shoots (leaves and stems) were subjected to steam distillation-extraction for 4 h according to the protocol given by Rao et al. (2005) with slight modifications. The condensate obtained from distillation was collected and divided into sub-samples. Each sub-sample was mixed with 100 ml hexane to trap the dissolved EO. EO obtained from the samples was dried over anhydrous sodium sulphate to make it moisture free. Quantity of EO was measured by calibrated burette. Percentage EO yield of tissue samples was calculated by the formulae given by Rao et al. (2005):

EO yield (%) = Amount of EO recovered (g) / Amount of crop biomass distilled (g) x 100

3.3. EO analysis

EO analysis was carried out by gas chromatography using Shimadzu GC 2014 equipped with flame ionization detector (FID) and an electronic pressure control (EPC) injector. Helium was used as the carrier gas with a flow rate 1.2 ml/min through Restek DB-5 capillary column (30 m long x 0.53 µm diameter and film thickness 0.5 µm). One µl oil sample was injected, diluted in hexane (0.05 ml of oil into 0.95 ml of GC standard grade hexane) and the samples were analyzed by GC. Analysis was programmed for 15 min at 60ºC, rising up to 240ºC at the rate of 5ºC/min. EO components were identified by comparing their retention times with those of authentic standards of linalool, 1,8- cineol, methyl cinnamte, methyl eugenol and camphor (CDH and Merck, India) run under the same conditions (Kovats, 1965). Concentration of each component in oil sample was calculated using the following equation (Lee et al., 2005).

Concentration (mg/g) = Weight of extract (without solvent) x GC peak area % 100 (mg) / Weight of plant material (g)

3.4. Trichome study

Micromorphological observations were carried out on fresh basil leaves by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). TEM was used to reveal ultrastructural details of trichomes.

3.4.1. *Light microscopy (LM):* Hand-peel mounts were prepared to study trichome density per unit leaf area on both adaxial and abaxial surfaces. For paraffin wax sectioning, plant material was fixed in FAA (formaldehyde: glacial acetic acid: ethanol, 1:1:18, v/v/v) for 24 h. For dehydration the fixed plant parts were passed through tertiary butyl alcohol (TBA) series. Embedding was done in paraffin wax following the procedure of O'Brien and McCully (1981). Wax blocks containing embedded material were trimmed and mounted on wooden blocks for sectioning with the help of Riechert rotary microtome. Sections of 8 µm thickness were cut, dewaxed and dehydrated through ethyl alcohol-xylene series and stained with 1% safranin and 1% astra blue combination. Mounting was done in DPX and observed under Nikon photomicroscope.

3.4.2. *Scanning electron microscopy (SEM):* Leaf segments were fixed in Karnovsky's fixative, buffered with 0.1 M sodium phosphate buffer at pH 7.4 for 6-8 h at 4ºC. After washing in the buffer the material was dehydrated in a graded ethanol series, critical point dried with CO_2, mounted in stubs and coated with a thin layer of gold (Serrato-Valenti et al., 1997). Sections were observed under LEO 435 VP scanning electron microscope at 15 KV.

3.4.3. *Transmission electron microscopy (TEM):* For ultrastructural investigation, leaves were trimmed and small pieces from margin up to midrib were fixed in Karnovsky's fluid, buffered with 0.1 M sodium phosphate buffer at pH 7.4 for 6-8 h at 4 ºC and post fixed in 1% osmium tetraoxide. After dehydration in ethanol series, the material was embedded in Epon-Araldite resin. Sections were conventionally stained with uranyl and lead citrate (Ascensão et al., 1997) and examined under Philips 200 transmission electron microscope at 80 KV.

3.5. Statistical analysis

Data were subjected to statistical analysis using software package SPSS 10.0 (Statistical Package for Social Sciences). One-way analysis of variance (ANOVA) followed by multiple comparison least significance difference (LSD) was employed to check the significance of the differences between the treatments at $p \leq 0.05$.

Conclusions

As in growth medium decreased both growth and biomass of aerial parts in *O. basilicum*. EO yield showed an up regulation at 10 and 50 mg/kg As, but it decreased at 150 mg/kg As. Linalool, the main component was augmented in all As treatments. Methyl cinnamate and camphor diminished under higher As concentrations. A strong positive correlation between trichome density and EO yield was noticed. Alteration in the structure and ultrastructure of glandular trichomes demonstrated that trichomes under As stress achieved precocious senescence, which further led to malfunctioning of secretory machinery. However, there is further need to carry out experiments in natural and controlled conditions to understand the exact mechanism by which the metabolic pathways are altered. Data provides an early indication that environmental pollutants like As affect the medicinal quality of *O. basilcum*. The development of optimized agricultural practices is essential for the sustainable cultivation of *O. basilicum* and adequate yield of EO.

References

Abedin M.J., and Meharg A.A., 2002, Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza*

sativa L.), Plant Soil, 243: 57-66
http://dx.doi.org/10.1023/A:1019918100451

Ascensão L., Marques N., and Pais M.S., 1997, Peltate glandular trichomes of *Leonotis leonurus* leaves: ultrastructure and histochemical characterization of secretions, Int. J. Plant Sci., 158: 249-258
http://dx.doi.org/10.1086/297436

Baatour O., Kaddour R., Aidi Wannes W., Lachaa M., and Marzouk B., 2010, Salt effects on the growth, mineral nutrition, essential oil yield and composition of marjoram (*Origanum majorana*), Acta Physiol. Plant., 32: 45-51
http://dx.doi.org/10.1007/s11738-009-0374-4

Bahl J.R., Garg S.N., Bansal R.P., Naqvi A.A., Singh V., and Kumar S., 2000, Yield and quality of shoot essential oil from the vegetative, flowering and fruiting stage crops of *Ocimum basilicum* cv Kusumohak, J. Med. Arom. Plant Sci., 22: 743-746

Begum F., Amin N., and Azad M.A.K., 2002, *In vitro* rapid clonal propagation of *Ocimum basilicum* L., Plant Tissue Cult., 12: 27-35

Ben Taarit M., Msaada K., Hosni K., Hammami M., Kchouk M.E., and Marzouk, B., 2009, Plant growth, essential oil yield and composition of sage (*Salvia officinalis* L.) fruits cultivated under salt stress conditions, Ind. Crops Prod., 30: 333-337
http://dx.doi.org/10.1016/j.indcrop.2009.06.001

Biswas S., Koul M., and Bhatnagar A. K., 2011, Effect of salt, drought and metal stress on essential oil yield and quality in plants, Nat. Prod. Commun., 10: 1559-1564

Bowers W.S., and Nishida R., 1980, Juvocimenes: potent juvenile hormones mimics from sweet basil, Science, 209: 1030-1332
http://dx.doi.org/10.1126/science.209.4460.1030

Cao H., Jiang Y., Chen J., Zhang H., and Huang W., 2009, Arsenic accumulation in *Scutellaria baicalensis* Georgi and its effects on plant growth and pharmaceutical components, J. Hazard. Mater., 171: 508-5013
http://dx.doi.org/10.1016/j.jhazmat.2009.06.022

Carbonell-Barrachina A.A., Aarabi M.A., DeLaune R.D., Gambrell R.P., and Patrick Jr, W.H., 1998, Arsenic in wetland vegetation: availability, phytotoxicity, uptake and effects on plant growth and nutrition, Sci. Total Environ., 217: 189-199
http://dx.doi.org/10.1016/S0048-9697(98)00195-8

Carbonell-Barrachina A.A., Burlo F., Burgos-Hernandez A., Lopez E., and Mataix J., 1997, The influence of arsenite concentration on arsenic accumulation in tomato and bean plants, Sci. Hortic., 71: 167-176
http://dx.doi.org/10.1016/S0304-4238(97)00114-3

Charles D.J., Joly R.J., and Simon J.E., 1990, Effect of osmotic stress on the essential oil content and composition of peppermint, Phytochemistry, 29: 2837-2840
http://dx.doi.org/10.1016/0031-9422(90)87087-B

Chaturvedi I., 2006, Effects of arsenic concentrations on growth and arsenic uptake and accumulation by rice (*Oryza sativa*) genotypes, Electron. J. Environ. Agric. Food Chem., 5: 1546-1552

Croteau R., and Johnson M., 1984, Biosynthesis of terpenoids in glandular trichomes, In: Rodriguez E., Healy P.L., and Mebta I. (eds.), Biology and chemistry of plant trichomes, Plenum, New York, pp.133-185
http://dx.doi.org/10.1007/978-1-4899-5355-1_7

Deef H.E.S., 2007, Copper treatments and their effects on growth, carbohydrates, minerals and essential oils contents of *Rosmarinus officinalis* L., World J. Agric. Sci., 3: 322-328

Elgayyar M., Draughon F.A., Golden D.A., and Mount J.R., 2001, Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms, J. Food Prot., 64: 1019-1024

Fahn A., 1988, Secretory tissues in vascular plants, New Phytol., 108: 229-257
http://dx.doi.org/10.1111/j.1469-8137.1988.tb04159.x

Fayiga A.O., Ma L.Q., and Zhou Q., 2007, Effects of plant arsenic uptake and heavy metals on arsenic distribution in an arsenic-contaminated soil, Environ. Pollut., 147: 737-742
http://dx.doi.org/10.1016/j.envpol.2006.09.010

Figueiredo A.C., and Pais M.S., 1994, Ultrastructural aspects of the glandular cells from the secretory trichomes and from the cell suspension cultures of *Achillea millefolium* L. ssp. *millefolium*, Ann. Bot. 74: 179-190
http://dx.doi.org/10.1006/anbo.1994.1107

Fischer R., Nitzan N., Chaimovitsh D., Rubin B., and Dudai N. 2011, Variation in essential oil composition within individual leaves of sweet basil (*Ocimum basilicum* L.) is more affected by leaf position than by leaf age, J. Agric. Food Chem., 59: 4913-4922
http://dx.doi.org/10.1021/jf200017h

Gadepalle V.P., Ouki S.K., Herwijnen R.V., and Hutchings T., 2008, Effects of amended compost on mobility and uptake of arsenic by rye grass in contaminated soil, Chemosphere, 72: 1056-1061
http://dx.doi.org/10.1016/j.chemosphere.2008.03.048

Ghosh A.K., Sarkar D., Bhattacharyya P., Maurya U.K., and Nayak D.C., 2006, Mineralogical study of some arsenic contaminated soils of West Bengal, India, Geoderma, 136: 300-309
http://dx.doi.org/10.1016/j.geoderma.2006.03.044

Gravano E., Tani C., Bennici A., and Gucci R., 1998, The ultrastructure of glandular trichomes of *Phillyrea latifolia* L. (Oleaceae) leaves, Ann. Bot., 81: 327-335
http://dx.doi.org/10.1006/anbo.1997.0562

Heikens A., 2006, Arsenic contamination of irrigation water, soil and crops in Bangladesh: risk implication for sustainable agriculture and food safety in Asia, Food and Agriculture Organization of United Nations Regional Office for Asia and the Pacific, Bangkok, Thailand, pp.1-38

Jirovetz L., Buchbauer G., Shafi M.P., and Kaniapady M.M., 2003, Chemotaxonomical analysis of the essential oil aroma compounds of four different *Ocimum* species from southern India, Eur. Food Res. Technol., 217: 120-124
http://dx.doi.org/10.1007/s00217-003-0708-1

Kovats E., 1965, Gas choromatographic characterization of organic substances in the retention index system, Adv. Chromatogr., 1: 229-247

Kumar B., Yaseen M., Patra N.K., Srivastava H.K., Naqvi A.A., and Ram P., 2004, Chemotypic variation in morphologically homogenous gene pool of Indian basil, Indian Perfum., 48: 289-297

Lee S.J., Umano K., Shibamoto T., and Lee K.G., 2005, Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties, Food Chem., 9: 131-137
http://dx.doi.org/10.1016/j.foodchem.2004.05.056

Liao X.Y., Chen T.B., Lei M., Huang Z.C., Xiao X.Y., and An Z.Z., 2004, Root distributions and elemental accumulations of Chinese brake (*Pteris vittata* L.) from As-contaminated soils, Plant Soil, 261: 109-116
http://dx.doi.org/10.1023/B:PLSO.0000035578.24164.fa

Marin A.R., Masscheleyn P.H., and Patrick W.H.Jr., 1992, The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration, Plant Soil, 139: 175-183
http://dx.doi.org/10.1007/BF00009308

Meharg A.A., and Hartley-Whitaker J., 2002, Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species, New Phytol., 154: 29-43
http://dx.doi.org/10.1046/j.1469-8137.2002.00363.x

Mehrag A.A., and Macnair M.R., 1990, An altered phosphate uptake system in arsenate-tolerant *Holcus lantus* L., New Phytol., 116: 29-35
http://dx.doi.org/10.1111/j.1469-8137.1990.tb00507.x

Morris J.A., Khettry A., and Seitz E.W., 1979, Antimicrobial activity of aroma chemicals and essential oils, J. Am. Oil Chem. Soc., 56: 595-603
http://dx.doi.org/10.1007/BF02660245

Murch S.J., Haq K., Ruoasinghe H.P.V., and Saxena P.K., 2003, Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum* L.), Environ. Exp. Bot., 49: 251-257
http://dx.doi.org/10.1016/S0098-8472(02)00090-4

Nasiri Y., Zehtab-Salmasi S., Nasrullahzadeh S., Najafi N., and Ghassemi-Golezani K., 2010, Effects of foliar application of micronutrients (Fe and Zn) on flower yield and essential oil of chamomile (*Matricaria chamomilla* L.), J. Med. Plants Res., 4: 1733-1737

O'Brien T.P., and McCully M.E., 1981, The study of plant structure: principles and selected methods, Termarcarphi Pyt. Ltd., Melbourne, Australia, pp.357

Politeo O., Jukica M., and Milosa M., 2007, Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil, Food Chem., 101: 379-385 http://dx.doi.org/10.1016/j.foodchem.2006.01.045

Prakash D.C., and Kardage B.A., 1980, Influence of sodium chloride and sodium sulfate salinities on photo synthetic carbon assimilation in pea nut, Plant Soil, 56: 201-207 http://dx.doi.org/10.1007/BF02205848

Prasad A., Singh A.K., Chand S., Chanotiya C.S., and Patra D.D., 2010, Effect of chromium and lead on yield, chemical composition of essential oil, and accumulation of heavy metals of mint species, Commun. Soil Sci. Plant Anal., 41: 2170-2186 http://dx.doi.org/10.1080/00103624.2010.504798

Rao B.R.R., Kaul P.N., Syamasundar K.V., and Ramesh S., 2005, Chemical profiles of primary and secondary essential oils of palmarosa (*Cymbopogon martini* (Roxb.) Wats var. *motia* Burk.), Ind. Crops Prod. 21: 121-127 http://dx.doi.org/10.1016/j.indcrop.2004.02.002

Rao E.V.S.P., Puttanna K., Rao R.S.G., and Ramesh S., 2007, Nitrogen and potassium nutrition of French basil (*Ocimum basilicum* Linn.), J. Spices Aromatic Crop., 16: 99-105

Raseetha V.S., Cheng S.F., and Chuah C.H., 2009, Comparative study of volatile compounds from genus *Ocimum*, Am. J. Appl. Sci**.**, **6:** 523-528 http://dx.doi.org/10.3844/ajassp.2009.523.528

Rashid H.A., Nath D.K., Hossain M., Khan M.U., Shah A.L., Saleque M.A., Rahman M.S., and Ghani M.A., 2004, Variation of arsenic content in groundwater with depth and river distance: GIS mapping. In: Shah Latif et al. (eds.), Arsenic in the food chain: assessment of arsenic in the water--soil--crop systems, BRRI, Gazipur, Bangladesh, pp.53-71

Reuveni R., Fleischer A., and Putievsky E., 1984, Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes, J. Phytopathol., 110: 20-22 http://dx.doi.org/10.1111/j.1439-0434.1984.tb00736.x

Rice E.L., 1979, Allelopathy---an update, Bot. Rev., 45: 15-109 http://dx.doi.org/10.1007/BF02869951

Salamon I., 2008, Chamomile biodiversity of the essential oil qualitative--quantitative characteristics, In: Sener, B. (ed.), Innovations in chemical biology, Springer Publishers, the Netherlands, pp.83-90

Serrato-Valenti G., Bisio A., Cornara L., and Ciarallo G., 1997, Structural and histochemical investigation of the glandular trichomes of *Salvia aurea* L. leaves, and chemical analysis of the essential oil, Ann. Bot., 79: 329-336 http://dx.doi.org/10.1006/anbo.1996.0348

Stancheva I., Geneva M., Hristozkova M., Boychinova M., and Markovska Y., 2009, Essential oil variation of *Salvia officinalis* (L.) grown on heavy metals polluted soil, Biotechnol. Biotechnol. Equip., 23: 373-376 http://dx.doi.org/10.1080/13102818.2009.10818442

Werker E., 1993, Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae---a review, Flavour Fragrance J., 8: 249-255 http://dx.doi.org/10.1002/ffj.2730080503

Wolff G., Pereira G.C., Castro E.M., Louzada J., and Coelho F.F., 2012, The use of *Salvinia auriculata* as a bioindicator in aquatic ecosystem: biomass and structure dependent on the cadmium concentration, Braz. J. Biol., 72: 71-77 http://dx.doi.org/10.1590/s1519-69842012000100009

Zhang W., Cai Y., Tu C., and Ma L.Q., 2002, Arsenic speciation and distribution in an arsenic hyperaccumulating plant, Sci. Total Environ., 300: 167-177 http://dx.doi.org/10.1016/S0048-9697(02)00165-1

Zheljazkov V.D., Craker L.E., and Xing B., 2006, Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil, Environ. Exp. Bot., 58: 9-16 http://dx.doi.org/10.1016/j.envexpbot.2005.06.008

Zheljazkov V.D., Jeliazkova E.A., Kovacheva N., and Dzhurmanski A., 2008, Metal uptake by medicinal plant species grown in soils contaminated by a smelter, Environ. Exp. Bot., 64: 207-216 http://dx.doi.org/10.1016/j.envexpbot.2008.07.003

Zheljazkov V. D., and Nielsen N.E., 1996, Studies on the effect of heavy metals (Cd, Pb, Cu, Mn, Zn and Fe) upon the growth, productivity and quality of lavender (*Lavandula angustifolia* Mill) production, J. Essent. Oil Res., 8: 259-274 http://dx.doi.org/10.1080/10412905.1996.9700612

International Journal of Plant and Environment
Vol. 4, No. 2 (July, 2018), 97-101

Looking at Neonicotinoid Insecticides: Environmental Perspective

Netra Bhandari and Monika Koul*

Department of Botany, Hansraj College, University of Delhi, Delhi-110007, INDIA

Publication Info

Article history:
Received: 22.02.2018
Accepted: 27.04.2018
DOI: 10.18811/ ijpen.v4i02.12

Key words:
Environment
Food crops
Human health
Insects
Neonicotinoids
New resembling nicotine

****Corresponding author:***
Dr. Monika Koul
Mob.: +91-98688997658
Email: drmkoul@gmail.com

Abstract

The recent "Global report on Food Crises" published by Food Security Information Network (FSIN, 2017) calls for intervention in methods and technologies to improve the quality and timeliness of food security and tackle the present food crises. India has a huge challenge to feed 1.32 billion and it is a daunting task. Droughts and other meteorological phenomena including, climate change, increase in pollution levels and spread of plant diseases and pests are some of the most common problems that continue to have an impact on food production. In order to manage crop diseases and reduce crop losses, low cost broad-spectrum insecticides have been synthesized. Farmers are using these both in situ and ex situ to save the crops and minimize the losses. However, all major pesticides have been found to have detrimental effects on social insects, and insects develop resistance to these after consistent and rampant use. Prolonged residence time of these in the environment also have harmful health implications and sometimes cause irreversible damage to human health. Therefore, time and again scientists are at look out for novel products and chemicals that can help in managing pests. The use and discovery of neonicotinoids proved to be a novel innovative method in diseases management of major crops. The newly discovered family of pesticides is attributed with various properties that are inherently different from other pesticides, and have the potential to kill a bouquet of crop pests including those that affect fruits, vegetables, fish and veterinary without conferring any resistance to them. However, the rampant use of neonicotinoids for crop protection has resulted in many unforeseen environmental problems. It is important to look for alternatives for the existing ones to tackle the human health problems. Scientists are also looking at decreasing the doses and treatment methods to reduce the impact on agro-ecosystems. A paradigm shift is required in crop management practices and indiscriminate use has to be stopped. Discovery of new generation neonicotinoids with interdisciplinary approach is one of the ways to tackle the present problems and meet the future challenges. Though, there are evidences that these novel formulations show developmental neurotoxicity, the dosage and frequency of applications show variable response. Research in this field is further required to substantiate the evidences of these insecticides to be safe to environment.

1. Introduction

The population in India is growing at a fast pace and the projections state that India may surpass China in population in the next few decades. The increase in population suggests that the production of food resources should also increase substantially to meet the growing demands (FAO: Global agriculture towards 2050). However, the potential to raise crop yields with the existing technologies does not seem to be possible. Therefore, focus should be on some other domains such as saving of crop losses on and off field from pests.Management of crop losses on account of spread of diseases caused by insect pests both in the field and post-harvest interests scientists all across the globe.

Agrochemical industries are witnessing spur in investments as well as profits via increased outreach and job markets (Jeschke *et al*., 2011). India is now the second largest producer of pesticides in Asia (Nollet *et al*., 2016). We also stand third in terms of pesticide use and are one of the largest global pesticide consumer nations of the world (Nollet *et al*., 2016). Some of these pesticides have also found a huge market base in many parts of South East Asia, especially neighbouring countries of Bangladesh, Pakistan, Nepal and Sri Lanka. However, there are lots of negative implications of excessive use of these on environment, biodiversity and human health. This aspect has been neglected and ignored for a long time, and is of great concern.

Among the most popular pesticides, are the insecticides which have seen many transitions from the first plant based nicotine insecticides in 1690 to synthetic organic forms like organophosphates, methylcarbamates, organochlorines and pyrethroids from 1940s-1970s (Tomizawa and Casida, 2011). Resistance in the pests to these widely used insecticides in the late 1980s gave a head start to the newly introduced neonicotinoid insecticides that recorded unique success in turnover in the 1990s. The use of these has increased and these have captured markets reaching a share of 24% in many countries. Though, these new generation pesticides could overcome the draw backs of conventional agrochemicals that were being used all over the world, some of the concerns are raised regarding their long persistence period. The present paper reviews the usage of neonicotinoids and the environmental and human concerns such as teratogenic impacts about these in India, especially when diseases like cancer and in-born errors of metabolism are being diagnosed on a large scale. Besides, these new generation pesticides are also now being implicated for loss of many invertebrate species especially useful insects and earthworms that are of enormous ecological and economic value.

2. Neonicotinoids

Neonicotinoids (novel insecticides resembling nicotine) are synthetic insecticides but are similar in action to nicotine, an alkaloid derived from Solanaceae, such as *Nicotiana* sps. These are primarily analogues of natural organic compound nicotine and also act in the similar manner on nervous tissues of insects and neuro-receptors. Due to their role in incapacitating insects and potential use as novel natural compounds in insect control, these are an in huge demand. Neonicotinoids have broad spectrum use and help in containing variety of insect pests and can be used for many crop species. The pharmacore moiety containing nitromine, nitromethylene, or cyanimide determines the insecticidal potency and selectivity of neonicotinoids (Matsuda *et al*., 2005). Presently, neonicotinoids are represented by many commercial compounds such asacetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethoxam and are available in the market and sold all over the world. Dinotefuran and sulfoxaflor are some new neonicotinoids which are synthesized on commercial scale in many parts of South East Asia especially, China (Shao *et al*., 2013). Another reason for their huge success is the versatile method of application on plants and the flexible nature of applications. Neonicotinoids can be put to use in various ways, for example in many places, these are mixed with water used for irrigation of vegetables. Their application in float system for tobaccos, seedling box application for rice, soil drenching method for citrus plants, application on trunks of apple trees, drench and drip application for coffee and grapevines, soil injections, bud injection for bananas are some other ways in which these are used. Application as seed treatments and soil injectionare the most common methodsof application and account for 60% of usage (Jescke *et al*., 2011). The method has high success rate for various crop species used in food industry. In USA from 2000 to 2012, virtually all neonicotinoids applied to maize, soybeans, and wheat were applied as seed treatments (Douglas and Tooker, 2015). Experimental analysis of effect of neonicotinoids on the leaf hopper (*Empoasca kerri*), a sucking pest on cowpea plants revealed that when seeds are treated in combination with foliar sprays, the plants acquire more resistance to the pests and combining the methods of treatment is more effective than either of the applications alone (Antu *et al*., 2017).

Moreover, they also have higher persistence and long residence time in the environment hence longer crop protection potential. Bonmatin *et al*. (2015) have comprehensively summarised the environmental fate and half-life of these insecticides in abiotic and biotic environments. Similarly, Jones *et al*. (2014) demonstrated that post application these stay in soil residues and may persist for several years, making them cost effective.

3. Mode of Action

Neonicotinoids are systemic in nature i.e. they are taken up by the plants through roots or leaves and then translocated to other plant parts. Thus, are among the most effective insecticides against sucking insect pests such as aphids, whiteflies, thrips, leaf and plant hoppers, some Lepidoptera and Coleopteran pests (Simon-Delso *et al*., 2015). In an inclusive summary, Elbert *et al*. (2008) have mentioned the use of the seven neonicotinoid insecticides (excluding sulfoxaflor) on the number of plants along with their specific pest target spectrum and the quantity of application as either foliar, soil or seed treatments. Some of the key crops mentioned are vegetables, pome and stone fruits, citrus, rice, cotton, corn, potato, sugar beet, oilseed rape, and soybean and many more.

Neonicotinoids target Acetylcholine receptors in the central nervous system of insects. Acetylcholine is a neurotransmitter, and the blockage of its receptors leads to overstimulation and paralytic condition in the insects. Thus, there is no cross-resistance to conventional insecticides such as organophosphates, pyrethroids, etc. (Vastrad, 2003). Unlike other insecticides, they are selectively toxic to the Insecta and reported to be relatively harmless to other mammals due to differences in properties and structure of subunits of nicotinic acetyl choline receptors (Tomizawa and Casida, 2003). This protects crops from an array of sap feeding insects (Nauen *et al*., 2008; Magalhaes *et al*., 2009; Bonmatin *et al*., 2015) and other agriculturally important crop pests (Elbert *et al*., 2008; Jeschke *et al*., 2011). Insects are exposed to multiple interacting environmental parameters and factors. There is also immense amount of diversity in the insects at populations, species and individual level. Social insects, such as bees, have been studied for their response to neonicotinoids but the studies are confined to lab experiments and the responses vary with the size of the bee, foraging behaviour and amount of the insecticide used. In many species, the foraging activity of individual bees was reduced. However, most of the social bees work in coordination in groups and studies have not been carried out at population level in the field. Studies are required that look at the research or cases at population level that link individual effects and reflect upon the synergism as well. However, a study conducted by Woodcock *et al.* (2017) on honey bees at multiple sites at Hungry, United Kingdom and Germany on oilseed rape crops suggested a varied effect on bee populations. There was a colony collapse observed in UK during the winter season and these persisted in environment for longer durations.

Literature survey suggests that the dosage of treatments is crop dependent. Concentrations in plant tissues and sap between 5 and 10 ppb (parts per billion) were sufficient to provide protection against pest insects (Castle *et al*., 2005; Byrne and Toscano, 2006). Toxicity studies carried out in 1,800 lab rats resulted in NOEL (No Observable Effect Level) of 100 ppm; hence neonicotinoids are very less toxic to mammals and can exit the body within 48 hours of ingestion (Fishel, 2005). Despite the immense potential of neonicotinoid insecticides against plant pests, agricultural yield in the developed countries have been modest in the past 20 years (Goulson, 2013). There is need for more studies comparing the effectiveness of neonicotinoids with other available crop protection measures like Integrated Pest Management (IPM) (Goulson, 2013).

4. Concerns

4.1. Human health

The studies on effect of neonicotinoids are largely carried out on insects. Sheets (2002) has summarized in his book that animals exposed to the neonicotinoid and imidacloprid results in tremors, hypothermia and impaired papillary function similar to that of exposure to nicotine. Reports published recently in leading journals suggest that these pesticides can affect the respiratory and nervous system. In a recent research Seltenrich (2017) reviewed the effect of neonicotinoids on humans and concluded that these compounds have shown an ability to bind to the most common receptor in nerve cells, cholinesterases nACRs in mammals. Change in the density of this neuroreceptor causes several central nervous system disorders such as Alzheimer's, Parkinson's, schizophrenia, and depression. The studies in laboratory animals suggest that these pesticides can affect hormones and their functioning thus affecting various metabolic processes in them. Therefore, the focus on effect of low dosage on endocrine effect of the chemical pesticides has now become crucial field of studies for scientists. Investigations are also on to study the effect on various enzymatic and metabolic pathways to decipher the role of these on humans.

4.2. Environment and biodiversity

Ecosystem services are gaining importance through economic valuation. In the year 2011, the total global ecosystem services were estimated to be $125 trillion/year (Costanza *et al*., 2014). This concept is now being widely used in decision making especially in cases of ecological risk assessment of the plethora of chemicals being developed every day. Due to their unique properties, neonicotinoids have entered and retained themselves in every sphere of the Earth. Their presence in soil, air and water has been documented even at places where their use is irrelevant.

Soil ecosystem services are largely driven by biological interactions. Neonicotinoid insecticides in the form of soil injections and release from seed coatings; pose a risk of harm to earthworms and other soil invertebrates as well as on microbes (Pisa *et al*., 2015) and are equally up taken by plants (Mullins, 1993). Their long persistence poses a relevant threat to soil ecosystem services (Chagnon *et al*., 2014). The effect thus can be detrimental as earthworms help in churning and aeration of soil that is important for the growth of plants.

Contamination of ground water is another negative consequence of application of pesticides on soil; depending on type of soil and concentration of pesticides in soil (Gupta *et al*., 2002; Huseth and Groves, 2014). Runoff from agricultural land, urban and semi-urban regions also contaminate the surface water causing negative impacts on aquatic organisms (Armbrust and Peeler, 2002).

A literature review conducted by Sánchez-Bayo *et al*. (2016) revealed that 22 studies have focussed on the effect of neonicotinoids (especially imidacloprid, thiacloprid and acetamiprid) and that there is no detailed study conducted to assess their impact on the entire ecosystem. The consequences of widespread water residues cause decline in the population of invertebrates and in turn starvation of insectivores. Thus, an impact on food chain can be detrimental to entire structure and functioning of the aquatic ecosystem.

Moreover, seed coating of insecticides which used to be considered as a safer option has shown increased release of insecticides in the air, which in turn affects non-target organisms. Greatti *et al*. (2003) stated that abraded insecticides settle on nearby flowering plants and are responsible for direct exposure to the pollinators. Therefore, the purpose for which they were introduced can fail and the pollinator loss will have to be addressed with some novel strategy.

High toxicity towards insect pests has increased the production and usage of neonicotinoids to many folds; however, their so called selective mode of action has not spared other non-target organisms. There have been constant debates between their effectiveness and consequent harm on non-target organisms. The massive loss of bee population is now linked to the presence of insecticides in pollen and bee bread (Bonmatin *et al*., 2015).

Since their water solubility is relatively higher than other pesticides, they are easily taken up by the plants and get translocated through both xylem and phloem to all plant parts. This systemic property which is very advantageous in controlling sap sucking pests, is equally harmful for pollinators like honey bees which depend on nectar and pollen from the crop plants. The lethal dose (LD50) value for oral/contact toxicity of neonicotinoid insecticides based on laboratory estimates show that imidacloprid, dinotefuran, clothianidin and thiamethoxam are considered highly toxic to honey bees while acetamiprid and thiacloprid are moderately toxic (Hopwood *et al*., 2016). The risk of exposure to non-target organisms, however also depends on crop type, method of application and the time period following the treatment.

Residues and high concentrations of neonicotinoids are also found in guttation droplets (Tapparo *et al*., 2011). Similarly, bees using resins for their hives are exposed to pesticides used on sunflower plants (Pareja *et al*., 2011). Thus, pollen, nectar, guttation droplets, resins and fruits are direct contacts for a pollinator to get exposed to insecticides. This can lead to toxic deposits in honey used for pharmaceutical preparations or as food in the human diet.

A toxicity analysis of nine insecticides carried out in rice fields in Tamil Nadu, India revealed that thiamethoxam showed highest toxicity to *Trichogramma chilonis* (polyphagous wasps) which is a natural enemy to important rice pests and often used in Ecological Pest Management, an approach that uses intrinsic strength of natural processes to reinforce the biological control of pests (Preetha *et al*., 2009). Similarly, thiacloprid was found to be inappropriate and ineffective for application on eggplants as well as for human consumption (Saimandir *et al.*, 2009). Another laboratory analysis deciphered the impact of imidacloprid on development of chick embryos. The dosage ranged from 5 to 50μg and resulted in growth retardation leading to failure in retraction of yolk sacs, defective limbs and neural tube (Hussein *et al*., 2014).

Recently cases of resistance towards neonicotinoids have also come in the limelight more specifically to the use of imidacloprid. Pests including *Amrasca biguttula biguttula* (cotton leaf hopper) and *Aphis gossypii* (cotton aphid) have shown resistance to imidacloprid (Wang *et al*., 2001; Kshirsagar *et al*., 2012). Gorman *et al*. (2008) illustrated how rice brown planthopper collected from India, China, Indonesia, Malaysia, Thailand and Vietnam showed reduced efficacy to imidacloprid. Continuous use of neonicotinoids has resulted in their resistance among pests belonging to various insect classes.

A study conducted in Netherlands has shown a decreasing trend in insectivorous bird population with the increase in use of imidacloprid insecticides (Hallmann *et al*., 2014). Pisa *et al*. (2015) documented extensively about the effect of neonicotinoids on non-target organisms including both terrestrial and aquatic invertebrates. They also comprehend the fact that although the plethora of literature has focussed on the negative impacts of neonicotinoids on a few organisms, it is enough to support their polluting potential and adverse biological and ecological impacts on organisms in terrestrial, aquatic, marine and benthic habitats.

6. Conclusions

Neonicotinoids are systemic insecticides which have proven to be very effective against many crop pests and have gained huge market success. However, as has happened with all insecticides, neonicotinoids also have harmful effects on non-target organisms. While in the developed countries a tremendous amount of literature is available about their sectorial usage, applications, and effect on non-target organisms like honey bees and on ecosystem services; there is a dearth of informationon their usage in countries like India. Only a few selected studies have focussed on the persistence of particular neonicotinoids in soil. Thiamethoxam and imidacloprid are two such insecticides of which effect on particular pests; and development of resistance has been studied.

There are some knowledge gaps that exist in neonicotinoid research in India. Assessment of state wise utilisation of insecticides and evaluation of persistence of neonicotinoids in all environmental media with special emphasis on aquatic ecosystems is something that needs immediate attention. Field related studies to understand the effect of metabolites and residues on non-target organisms, and finally to understand the effect of all insecticides acting together in the environment is much desired.

Furthermore, experiments need to be carried out in field conditions with substitutes or fortified neonicotinoids so that the latest strategy of Ecological Pest Management and development ofsafe pest control measure is the adopted in large scale farm holdings so that the natural forces and processes of pest regulation come into play. This can be reinforced by conserving the biological diversity of the regions. Usage of safe control measures, dosage and frequency at which these should be applied has to be standardized for various crop species.

Acknowledgements

Authors thank University Grants Commission for the funding in the form of MRP-UGC Project.

References

Antu, M., Korat, D. and Sreelakshmi, P. 2017. Management of leaf hopper infesting cowpea by seed treatment and foliar spray with neonicotinoid insecticides. *International Journal of Farm Sciences* **6**(4):95-103.

Armbrust, K.L. and Peeler, H.B. 2002. Effects of formulation on the run-off of imidacloprid from turf. *Pest Management Science* **58**(7):702-706.

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A. and Noome, D.A. 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Science and Pollution Research* **22**(1):35-67.

Byrne, F.J. and Toscano, N.C. 2006. Uptake and persistence of imidacloprid in grapevines treated by chemigation. *Crop Protection* **25**(8):831-834.

Castle, S.J., Byrne, F.J., Bi, J.L. and Toscano, N.C. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. *Pest Management Science* **61**(1):75-84.

Chagnon, M., Kreutzweiser, D., Mitchell, E.A., Morrissey, C.A., Noome, D.A. and Van der Sluijs, J.P. 2015. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental Science and Pollution Research* **22**(1):119-134.

Costanza, R., de Groot, R., Sutton, P., Van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S. and Turner, R.K. 2014. Changes in the global value of ecosystem services. *Global Environmental Change* **26**:152-158.

Denholm, I., Cahill, M., Dennehy, T.J. and Horowitz, A.R. 1998. Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **353**(1376):1757-1767.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C.H., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., Van der Sluijs, J.P., Whitehorn, P.R. and Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research* **22**(1):5-34.

Douglas, M.R. and Tooker, J.F. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and pre-emptive pest management in US field crops. *Environmental Science and Technology* **49**(8):5088-5097.

Elbert, A., Haas, M., Springer, B., Thielert, W. and Nauen, R. 2008. Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science* **64**(11):1099-1105.

FSIN 2017. Food Security Information Network (FSIN) Report. Resource on Food Security Analysis, FAO.

Sánchez-Bayo, F., Goka, K. and Hayasaka, D. 2016. Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems. *Frontiers in Environmental Science* **4**(71):1-14.

Fishel, F.M. 2005. Pesticide Toxicity Profile: Synthetic Pyrethroid Pesticides. The Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, PI-54, pp. 1.4.

Georghiou, G.P. and Mellon, R.B. 1983. Pesticide Resistance in Time and Space. In: Georghiou G.P. and Saito, T. (Eds.), *Pest Resistance to Pesticides*. Springer, Boston, MA, pp. 1-46.

Gorman, K., Liu, Z., Denholm, I., Brüggen, K. U. and Nauen, R. 2008. Neonicotinoid resistance in rice brown plant hopper, *Nilaparvata lugens*. *Pest Management Science* **64**(11):1122-1125.

Goulson, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology* **50**(4):977-987.

Greatti, M., Sabatini, A. G., Barbattini, R., Rossi, S. and Stravisi, A. 2003. Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing: Preliminary results. *Bulletin of Insectology* **56**:69-72.

Gupta, S., Gajbhiye, V.T. and Agnihotri, N.P. 2002. Leaching behavior of imidacloprid formulations in soil. *Bulletin of Environmental Contamination and Toxicology* **68**(4):502-508.

Hallmann, C.A., Foppen, R.P., van Turnhout, C.A., de Kroon, H. and Jongejans, E. 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* **511**(7509):341.

Hopwood, J., Code, A., Vaughan, M., Biddinger, D., Shepherd, M., Black, S.H., Lee-Mäder, E. and Mazzacano, C. 2016. *How Neonicotinoids can Kill Bees.* Xerces Society for Invertebrate Conservation, Portland, pp. 1-70.

Huseth, A.S. and Groves, R.L. 2014. Environmental fate of soil applied neonicotinoid insecticides in an irrigated potato agroecosystem. *PLoS One* **9**(5):e97081.

Hussein, M., Singh, V., Sethi, R., Singh, A.K. and Hassan, M.A. 2014. Study on embryonic effects of neonicotinoid insecticide on chick embryos. *Journal of the Anatomical Society of India* **63**(2):125-129.

Jeschke, P., Nauen, R., Schindler, M. and Elbert, A. 2011. Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry* **59**(7):2897-2908.

Jones, A., Harrington, P. and Turnbull, G. 2014. Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years. *Pest Management Science* **70**(12):1780-1784.

Kshirsagar, P., Burgos, R.P., Jang, J., Lidozzi, A., Wang, F., Boroyevich, D. and Sul, S.K. 2012. Implementation and sensorless vector-control design and tuning strategy for SMPM machines in fan-type applications. *IEEE Transactions on Industry Applications* **48**(6):2402-2413.

Magalhaes, L.C., Hunt, T.E. and Siegfried, B.D. 2009. Efficacy of neonicotinoid seed treatments to reduce soybean aphid populations under field and controlled conditions in Nebraska. *Journal of Economic Entomology* **102**(1):187-195.

Matsuda, K., Shimomura, M., Ihara, M.,Akamatsu, M. and Sattelle, D.B. 2005. Neonicotinoids show selective and diverse actions on their nicotinic receptor targets: Electrophysiology, molecular biology, and receptor modeling studies. *Bioscience, Biotechnology, and Biochemistry* **69**:1442-1452.

Mullins, J.W. 1993. Imidacloprid: A new nitroguanidine insecticide. In: Duke, S.O., Menn, J.J. and Plimmer, J.R. (Eds.), *Pest Control with Enhanced Environmental Safety*, ACS Symposium Series, ACS Publication, Vol. 13, 183-198.

Nauen, R., Reckmann, U., Thomzik, J. and Thielert, W. 2008. Biological profile of spirotetramat (Movento®)-a new two-way systemic (ambimobile) insecticide against sucking pest species. *Bayer Crop Science Journal* **61**(2):245-278.

Nollet, L.M.L. and Rathore, H.S. 2016. Handbook of pesticides: Methods of pesticide residues analysis. CRC Press, Boca Raton, Florida, pp. 1-197.

Pareja, L., Colazzo, M., Pérez-Parada, A., Niell, S., Carrasco-Letelier, L., Besil, N. and Heinzen, H. 2011. Detection of pesticides in active and depopulated beehives in Uruguay. *International Journal of Environmental Research and Public Health* **8**(10):3844-3858.

Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M. and Morrissey, C.A. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental Science and Pollution Research* **22**(1):68-102.

Preetha, G., Stanley, J., Suresh, S., Kuttalam, S. and Samiyappan, R. 2009. Toxicity of selected insecticides to *Trichogramma chilonis*: Assessing their safety in the rice ecosystem. *Phytoparasitica* **37**(3):209-215.

Sagar, D. and Balikai, R.A. 2014. Insecticide resistance in cotton leafhopper, *Amrasca biguttula biguttula* (ISHIDA): A review. *Biochemical and Cellular Archives* **14**:283-294.

Saimandir, J., Gopal, M. and Walia, S. 2009. Risk assessment of thiacloprid and its chemical decontamination on eggplant, *Solanum melongena* L. *Pest Management Science* **65**(2):210-215.

Sánchez-Bayo, F., Goka, K. and Hayasaka, D. 2016. Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems. *Frontiers in Environmental Science* **4**:71.

Seltenrich, N. 2017. Catching up with popular pesticides: more human health studies are needed on neonicotinoids. *Environmental Health Perspectives* **125**(2):A41-A42.

Shao, X., Swenson, T.L. and Casida, J.E. 2013. Cycloxaprid insecticide: Nicotinic acetylcholine receptor binding site and metabolism. *Journal of Agricultural and Food Chemistry* **61**(33):7883-7888.

Sheets, L.P. 2002. The Neonicotinoid Insecticides. In: Massaro, E.J. (Ed.), *Neurotoxicology Handbook*, Oxford, UK, Humana Press Inc, Vol. 1, pp. 79-87.

Tapparo, A., Giorio, C., Marzaro, M., Marton, D., Soldà, L. and Girolami, V. 2011. Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. *Journal of Environmental Monitoring* **13**(6):1564-1568.

Tomizawa, M. and Casida, J.E. 2003.Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology* **48**:339-364.

Tomizawa, M. and Casida, J.E. 2011. Neonicotinoid insecticides: highlights of a symposium on strategic molecular designs. *Journal of Agricultural and Food Chemistry* ***59***(7):2883-2886.

Vastrad, A.S. 2003. Neonicotinoids-Current success and future outlook. *Pestology* **27**:60-63.

Wang, K.Y., Liu, T.X., Jiang, X.Y. and Yi, M.Q. 2001.Cross-resistance of *Aphis gossypii* to selected insecticides on cotton and cucumber. *Phytoparasitica* **29**(5):393.

Woodcock, B.A., Bullock, J.M., Shore, R.F., Heard, M.S., Pereira, M.G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S. and Pywell, R.F. 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science* **356**:1393-1395.

Heavy metal uptake and contamination in medicinal plants: hype or reality!

Nikita Sharma[1] and Monika Koul[2]
[1]Department of Botany, University of Delhi, Delhi- 110 007
[2]Hansraj college , Department of Botany , University of Delhi – 110 007
drmkoul@gmail.com

Introduction

According to WHO 80% of the world population use herbal medicines for curing various diseases. Serious concerns are being raised regarding the use of allopathic drugs.. Resistance to these drugs is increasing and these also have proven side effects. Therefore, scientists are looking at the repository of medicinal plants for drug discovery. Reports suggest that 25% of modern medicines are derived from plants. However, most of the medicinal plants are growing in wild and in wastelands. These plants are collected from various places and no special methods are used by practitioners to test the safety aspects. *Solanum nigrum*, *Withania somnifera*, *Plumbago zeylanica*, and many other plants are used for treatment of various ailments. Entire plants and their parts such as roots, leaves and fruits are also used ethano-botanically by the local people. It has been deciphered that many of these plants are metal accumulators. As the pollution level is increasing all across the habitats on account of various anthropogenic activities, heavy metal concentration above permissible limits in the substratum is reported. It is important to assess the uptake of heavy metals in plants and their parts. The study is being undertaken at various sites in Delhi, Haryana and Uttar Pradesh to assess the safety and efficacy of the drugs derived from the selected medicinal plants.

Withania somnifera

Solanum nigrum

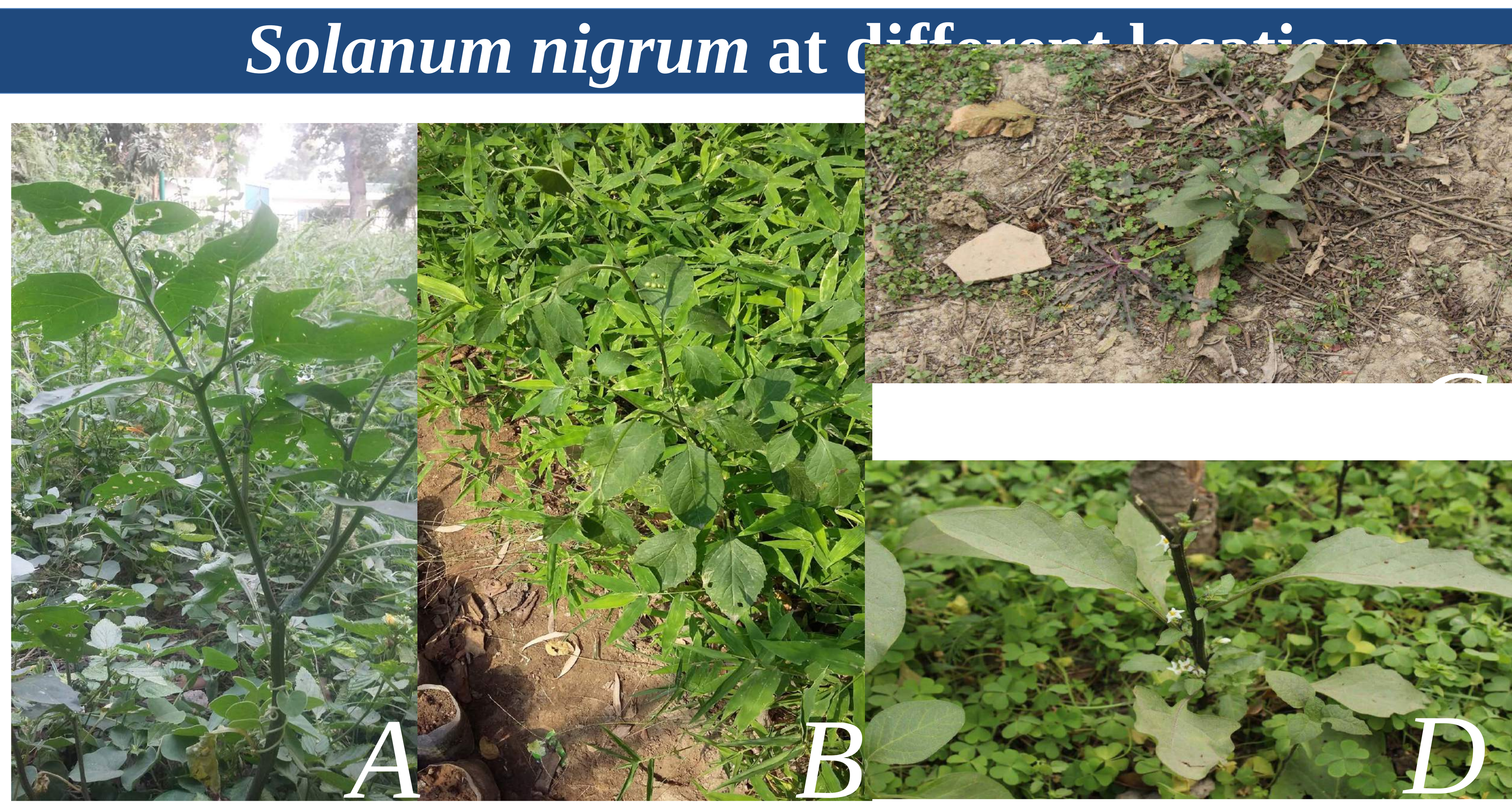
Salvia officinalis

Plumbago zeylanica

Research Aim

- To collect plants of *Withania* and *Solanum* from various sites (polluted industrial areas, city wastelands, roadside and sewage) disposal sites
- Analysis and estimation of heavy metal pollutants in plant samples by using different techniques
- Comparative study of uptake of metals in various plant parts in selected plants and the soil.

Solanum nigrum at different locations



A: Kundli Industrial Area near Sonipat, Haryana, B: Kamla Nehru Ridge, near University of Delhi, Delhi, C: Near Inorganic lab, Department of Chemistry, University of Delhi, Delhi, D: Botanical Garden, Department of Botany, University of Delhi, Delhi

Methodology

Collection of plant samples
↓
Oven drying of the samples
↓
Acid Digestion
↓
Samples analysis using AAS
↓
Results

Results and Discussion

Table1: Amount of Lead and Zinc in fruits and leaves of *Solanum nigrum*

S.No.	Area	Lead[a]($\mu g g^{-1}$) (Fruits)	Lead[a]($\mu g g^{-1}$) (Leaves)	Zinc[a]($\mu g g^{-1}$) (Fruits)	Zinc[a]($\mu g g^{-1}$) (Leaves)
1	Kundli industrial area near Sonipat, Haryana	16.40	16.92	441.08	428.45
2	Kamla Nehru Ridge, near University of Delhi, Delhi	13.22	17.44	435.02	516.02
3	Botanical Garden, Department of Botany, University of Delhi, Delhi	13.51	6.66	461.71	445.83

[a] using Atomic Absorption Spectroscopy

Graphical Representation

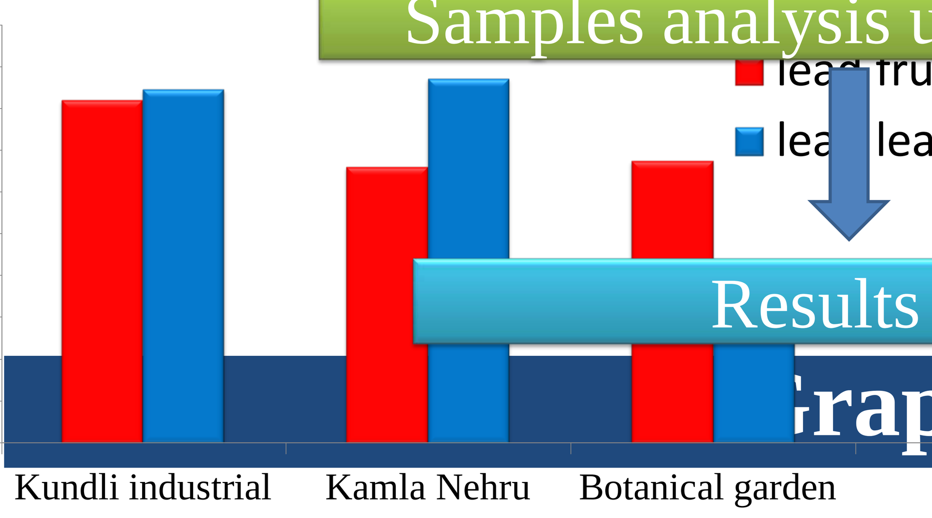


Fig. 1 Graphical representation of amount of lead in fruits and leaves

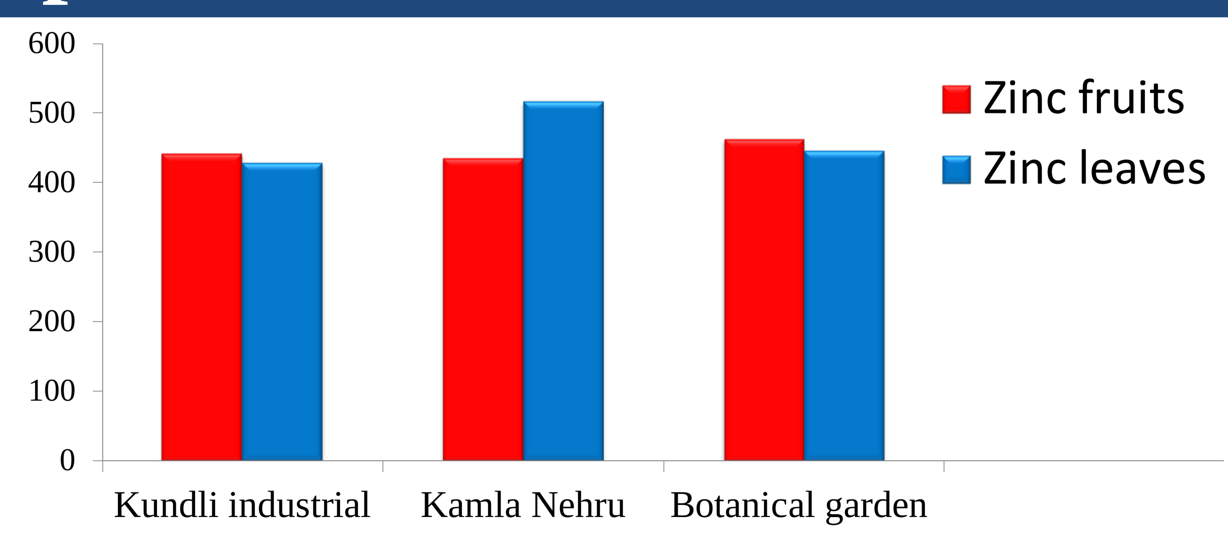


Fig. 2 Graphical representation of amount of Zinc in fruits and leaves

References

•WHO. Quality Control Methods for Medicinal Plant Materials 1998, 62-63
•Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, Vol.6. Central Drug Research Institute, New Delhi, (1998)

Acknowledgement: We acknowledge the financial support provided by UGC for the present study.