

Imidacloprid residues in cotton guttation fluid, pollen and soil when applied as seed dressings

K Rolania^{a#}, A Janu^b, S Ahlawat^c and SS Yadav^d



Summary

Guttation fluid and pollen are possible pathways where pollinators may be exposed to systemic insecticides. During the investigation, *Gossypium hirsutum* v. H 1098i seeds were coated with Gaucho (Imidacloprid 600 FS at 2.4 g a.i. kg⁻¹ seeds) and planted in the field. Guttation fluid, pollen, and soil samples were collected during the season and the samples were analysed by HPLC (High-Performance Liquid Chromatography). The guttation fluid and pollen were collected at three times after 50 per cent flowering. Soil samples were collected before sowing and at harvest. Method validation was achieved by performing a recovery experiment at two fortification levels of 0.05 and 0.10 mg kg⁻¹. The average recoveries obtained from guttation fluid, pollen and soil samples were above 85 per cent. The residues of imidacloprid in guttation fluid, pollen and soil in treated and untreated seed were below detectable levels.

JAE 2023, Vol 15

Received: 20 March 2023

Accepted: 15 June 2023

Published: 30 June 2023

<https://doi.org/10.58628/JAE-2315-121>

Associated Editor: Dr. MK Jat

Copyright © 2023 The Author(s). Published by Society for Agriculture and Arid Ecology Research (SAAER). This is an Open Access article under the Creative Commons Attribution License 4.0 (CC BY-NC-SA).



Keywords: *Gossypium hirsutum*, Guttation Fluid, Imidacloprid, Pollen and Seed Dressing

Introduction

Cotton is an important cash crop worldwide for the production of textile fibre, edible oil for human consumption, and feed for non-ruminant animals (Zang et al. 2014). One of the limiting factor in cotton production is damage from insect pests (Kalyan et al. 2017). Nearly 162 species of insect pests occur on cotton in India (Dhaliwal et al. 2010). Mainly two important groups i.e. bollworms and sucking insect pests, cause severe damage to the cotton. After the introduction of *Bt* cotton in India, the major three bollworms viz., *Helicoverpa armigera*, *Earias spp.* and *Pectinophora gossypiella* have attained a non-pest status on *Bt* cotton. As a result, sucking pest complex viz., *Bemisia tabaci*, *Amrasca biguttula biguttula*, *Thrips tabaci*, and *Aphis gossypii* are reported as major pests of cotton in India (Vennila et al. 2000; Kumar et al. 2009). The sucking pests are injurious as they suck the cell sap and transmit viral diseases. About 30-40 per cent yield losses occur in cotton annually from sucking pests (Haque 1991, Kumar et al. 2012a&b). Infestations may also cause deterioration in lint quality. Thus sucking insect pests are regularly monitored and insecticides are applied to manage them in the early stage of the crop.

A range of synthetic insecticides are used for controlling sucking pests worldwide. Insect pests have

developed resistance to many of the conventional insecticides like, chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Cui et al. 2016). In 1991, a new class of insecticides (i.e. neonicotinoids) was introduced to control insects in multiple crops and help in management of resistance against other classes of insecticides. Neonicotinoids are the nicotinic acetylcholine receptor (nAChR) agonists with good insecticidal activity and low mammalian toxicity (Tomizawa & Casida 2005; Ohno et al. 2009). Imidacloprid is a leading product among neonicotinoids and is used for plant protection applications. Due to its systemic nature, imidacloprid moves to different parts of the plant (root, leaf, flower etc). The declining population of honey bees and bumble bees have been suggested to be a result of neonicotinoids being translocated into pollen and nectar of foraging host (Schmuck et al. 2001; Blacquiere et al. 2012; Goulson 2013). The persistence of neonicotinoid residues in guttation fluid, pollen and soil can be toxic to pollinators. High residues were found in all crops at early growth stages, showing decline with increasing plant growth and age (Reetz et al. 2011). Guttation droplets are one of several possible water sources available for a honey bee colony and usually are only at a limited time. The pollen and nectar collection are regular requirement of the honey bees but the guttation liquid is utilised under the water scarcity and the risk is likely to decrease rapidly with distance of the colonies to treated plants and the availability of alternative water sources, e.g. ponds, dew, rivers. The residue content can determine the potential risk of uptake of guttation droplets from treated crops to satisfy water requirements. However, the use of guttation droplets as a water source for honeybees and it's arising impact on the loss of bee colonies is not yet clear (Wallner 2009). This paper presents the data of imidacloprid residue applied as a seed treatment in

^aAssistant Scientist, Department of Entomology, CCS Haryana Agricultural University, Hisar; krishna@hau.ac.in, +91 9468192822

^bBusiness Development Analyst, UPL SAS Limited, Bandra, Mumbai; arun.janu@upl-ltd.com, +91 9416613998

^cAssistant Chemist, Department of Entomology, CCS Haryana Agricultural University, Hisar; sushilahlawat08@gmail.com, +91 9416342952

^dAssistant Director (Plant Protection), Directorate of Research, CCS Haryana Agricultural University, Hisar; surinderyadav@rediffmail.com, +91 8295697470

[#]Corresponding author K Rolania, E-mail: krishna@hau.ac.in

guttation fluid, pollen and soil in cotton crop.

Material and Method

This experiment was executed at Research Area of Cotton Section, CCS Haryana Agricultural University, Hisar (India), during *kharif* (season) 2015 on *Gossypium hirsutum* var. H 1098i. Cotton seeds were treated with Gaucho (Imidacloprid 600 FS) at 2.4 g a.i. kg⁻¹ seeds. The treated seeds were allowed to dry for 24 hours under shade. The seeds were planted manually (in treated and untreated plot) at a spacing of 67.5 cm between the row and 30 cm between plants at 4-5 cm depth. The crop was sown on May 25, 2015 in plot size of 10x10M in three replications. The field soil type was sandy loam having 0.31 per cent organic carbon and pH-7.9. The flood irrigation system was used for watering the crop in which water is delivered to the field by simple flows over the ground through the crop. This irrigation method helps in maintaining the sufficient moisture in the soil.

Sampling: Guttation is the exudation of active or passive excretion of xylem liquid in the form of droplets on specific plant locations. Guttation drops usually appear on the tips and along the edges of the leaves. Guttation drop samples were collected early morning between 5 to 6 am after 50 per cent flowering at 70, 85 and 100 days after sowing. A total of 90 samples were drawn from three replication of both the treatments on all three stages (70, 85 and 100 days after sowing) with the help of a clinical syringe (suction tube). The sample liquid is directly transferred from the syringe into Eppendorf tube. Samples were immediately transferred to a -4°C freezer and were kept frozen until analysis. Pollen from cotton flowers was collected at 55, 70, 85 and 100 days after sowing. Matured flower buds which were ready to open the next day, as determined by the size and length of calyx tube, were chosen for pollen collection. To collect pollen, the bud was held between the thumb and index finger of the left hand and fine forceps were used to detach the pollen into a vial with ethyl alcohol gently. Soil samples (3 samples from unit area) of 500 g were collected by an auger (diameter 6 cm) at pre-sowing and another one at harvest. For soil sampling the field was divided into different homogenous units and surface litter was removed from the sampling spot. Auger was steered to a depth of 22 cm and soil sample was drawn and placed in a clean container.

Analytical Standard: The analytical reference standards of imidacloprid 600 FS (purity 99.8% w/w) was supplied by Bayer Crop Science Limited, India. Acetic acid, acetonitrile, ammonium hydroxide, celite, dichloromethane, ethanol, hexane, methanol, sodium carbonate of HPLC grade and activated charcoal were obtained from Merck India Limited.

Preparation of Calibration Solutions: A composite stock solution of the analytes was prepared in HPLC grade acetonitrile by suitably mixing the aliquots of analytical standards of imidacloprid. Calibration solutions were prepared by diluting the stock solution using mobile phase in the range 0.01 to 1.0 µg ml⁻¹ using serial dilution technique.

Instrumentation: An Agilent® prominence High-Performance Liquid Chromatography (HPLC) equipped

with Diode array detector (DAD) detector (λ_{max} – 250 nm for imidacloprid) was used for the quantification of residues of imidacloprid. A ZORBAX® C-18 Column of 4.6 i.d. and 250 mm length of 5 µm particle size was used to separate imidacloprid. The peaks of imidacloprid were separated using a mobile phase containing the mixture of 300 ml of acetonitrile and 700 ml of distilled water, with a programmed flow rate of 0.5 ml per min and the injection volume was 20 µl at a 3.34 min retention time. Extraction and clean up of residues in Guttation Fluid: The guttation fluid samples were diluted with 50 per cent water-methanol solution.

Extraction and clean up of residues in pollen: Ten grams of pollens were put in 20 ml of 75 per cent ethyl alcohol solution (75:25 Ethyl Alcohol: Water) and mixed for 1 min. It was rinsed 3 times with 5 ml of 75 per cent ethyl alcohol and centrifuged at 3600 rpm for 5 min. The extracted phases were collected in a flask and repeated for a second time. The recovered extracted phases were evaporated with a rotary evaporator and made the final volume of 5 ml in water. Ten millilitres of buffer (Na₂CO₃/CH₃COOH) (pH=7) was added in the flask and it was rinsed with 20 ml of dichloromethane for two times. The lower phase i.e. the organic phase was collected and concentrated and two ml of acetonitrile was used for dilution. Two ml of hexane was used to dilute oily residue and treated ultrasonically for 5 min. One millilitre of 50 per cent ACN (50:50 Acetonitrile: Water) was added to the residue, and centrifuged for 5 min at 4000 rpm. The upper phase was pulled out and shifted into 1.5-ml Eppendorf tubes and for 5 min centrifuged at 1200 rpm. A total of 25 µL was injected in HPLC.

Extraction and cleaning up of soil residues: 20 g of air-dried soil sample was mixed with 100 ml of 0.05 per cent methanol/ NH₄OH (3/1) solution for 1 minute and added Celite (2 g). Mix 20 g of air-dried soil sample with 100 ml of 0.05 per cent methanol/ NH₄OH (3/1) solution for 1 min and add Celite (2 g). The mixture was filtered on a glass frit containing 2 g of wet Celite with methanol in a vacuum condition. The extract was dried by vaporized at 50 °C with a rotary evaporator and flask was swilled with 15 ml of dichloromethane. The extract was again vaporized on a hot sand-bath and residue was dissolved in 1 ml of 50 per cent ACN (50:50 Acetonitrile: Water) using ultrasonication in sealed tubes. The extract was centrifuged for 2 minutes at 10000 rpm in 1.5-ml Eppendorf tubes and injects the volume of 20 µL in HPLC.

The soil samples were processed by using the method of Kumari et al. (2008). Representative soil samples (15 g) were thoroughly mixed with activated charcoal (0.3 g) and Florisil (0.3 g). The mixture was packed compactly in a column made up of glass in between two layers of anhydrous sodium sulphate each of 10 g and eluted with 125 ml of acetonitrile at a flow rate of 2–3 ml per minute. The obtained elute was concentrated by using rotary vacuum evaporator, and the final volume (3 ml) was made in acetonitrile for further analysis on HPLC.

Method validation: The method was validated by studying specificity, linearity and recovery. Specificity of

the method was checked by injecting all the control sample (cotton guttation fluid, pollen and soil) extracts and treated samples, diluting solvents, extraction solvent, acetonitrile and mobile phase. Different known concentrations 0.01 to 1.0 $\mu\text{g ml}^{-1}$ of imidacloprid were injected to check linearity of the method. The calibration solutions were prepared by diluting the stock solution using mobile phase. After injecting the calibration standard solutions the peak area was computed. The regression equation was determined by plotting the concentration of the standards injected against the area observed. The representative chromatogram is presented in Figure 1.

Recovery, limit of quantification and repeatability: Two known concentrations (0.05 and 0.010 mg kg^{-1}) of imidacloprid standard were added in the sample of cotton guttation fluid, pollen and soil sample to check the method's recovery. After fortification of the standard solution, the homogenized samples were extracted and subsequently analysed for imidacloprid residues. Three replicate determinations and two control samples were made at each fortification level. Based on the recovery, the limit of quantification was established. The method's repeatability was checked by calculating the relative standard deviation value for the area obtained from the individual measurements.

Result and Discussion

The linearity of the calibration curve was studied using imidacloprid standard solutions within the range of 0.01 to 1.0 $\mu\text{g ml}^{-1}$. All runs were repeated three times and the response of detector was obtained at λ_{250} in terms of peak areas. By plotting concentrations of imidacloprid in μg versus average peak area gives calibration curve as presented in Fig. 2. The response was found to be linear with correlation coefficient (R^2) = 0.993 and regression equation as $Y = 136.17X + 41.11$. The 0.01 μg concentration was determined as limit of quantification (LOQ) for imidacloprid. The validity of this method in guttation fluid, pollen and soil samples was checked by the conducting recovery experiments by fortifying the control samples at 0.05 and 0.010 mg kg^{-1} levels in triplicate. The mean recoveries for each matrix ranged from 85.23 to 82.10 per cent with relative standard deviation (RSD) below 10 per cent (Table 1). Analysis of cotton guttation fluid samples, collected three times at 15 days interval after 50 per cent flowering shows imidacloprid to below detectable level (BDL) of 0.01 $\mu\text{g g}^{-1}$ in dressing of seed (Table 2) with Gaucho (imidacloprid 600 FS). The present results are not in alignment with the finding of Tapparo et al. (2011) where they found imidacloprid (346 mg L^{-1}) above the lethal doses for bees in guttation fluid of corn seeds coated with insecticides. This may be due to time difference between sample collection and date of sowing. In our study cotton

samples are collected after 50 per cent flowering while in corn the samples are collected at 15-20 days after the seedling emergence. As Sur & Stork (2003) reported that imidacloprid showed good acropetal translocation (excellent xylem mobility) in comparison to basipetal translocation (negligible phloem mobility). They also found that the cotton plant uptake 4.9 per cent of applied insecticide up to the harvest.

The amount of residues in guttation droplets depends on the crop and its growth stage, the properties of the active substance, the amount of active substance per seed and other factors (Schenke et al. 2011). Highest residues were found in all crops at younger growth stages, showing decline with increasing plant age and growth stage (Schenke et al. 2010; Reetz et al. 2011).

The pollen samples of cotton flower collected three times at 15 days interval for analysis and found the imidacloprid below detectable level i.e. 0.01 $\mu\text{g g}^{-1}$. Helal et al. (2005) and Choudhary & Sharma (2008) conducted an experiment on mustard and treat the seed with Imidacloprid at 21 g a.i. kg^{-1} seed. They neither found any residue of imidacloprid in nectar nor in pollen samples at the time of sampling (i.e. 50% of flowering). These findings are in good agreement with Dikshit et al. (2002) who was also unable to observe any residues of imidacloprid at 60th day of sowing in treated seeds of okra. Kamel (2012) measured levels of neonicotinoid residues in nectar and pollen from a pumpkin crop treated at different timings and application methods. Foliar-applied treatments and chemigation insecticides applied through drip irrigation during flowering resulted in the highest residues of parent insecticide and metabolites. The lowest levels of residues were detected in treatment regimens involving applications of insecticides at planting, as seed dressing, transplant water treatment, or bedding tray drench.

Imidacloprid residues in soil samples collected at pre-sowing and at harvest of cotton were below detectable levels (Table 2). Similar results were reported by Pandiselvi et al. (2010), who analyzed the cotton lint, seed, oil and soil for the residue of spirotetramat, imidacloprid and its metabolite in cotton at harvest time. Although, Bonmatin et al. (2003) found that only 3 per cent of soil did not have imidacloprid ($<0.1 \mu\text{g g}^{-1}$) among 33 soil samples. This may be due to the variability of soil texture. Miles (1993) found that imidacloprid half-life varied from 27 to 229 days in soil and depended on presence or absence of ground cover, use of organic fertilizers and soil type. Scholz & Spiteller (1992) showed that imidacloprid degraded more rapidly under vegetation i.e. 48 days while 190 days without vegetation. In the present investigation no residues was present in guttation fluid, pollen and soil samples collected from control plots where untreated seeds were grown.

Table 1. Per cent recovery of imidacloprid in guttation fluid, pollen and soil

Fortification level (mg kg^{-1})	Guttation Fluid	Pollen	Soil
0.01	83.54 $\pm$ 1.98	86.63 $\pm$ 1.73	83.71 $\pm$ 1.52
0.05	88.5 $\pm$ 3.1	90.1 $\pm$ 2.2	87.8 $\pm$ 1.7
0.10	92.3 $\pm$ 1.8	98.5 $\pm$ 3.5	91.0 $\pm$ 4.3

^a Average of three replicates

Table 2. Residues of imidacloprid in guttation fluid, pollen and soil samples in mg kg⁻¹

Parameters	Residues* (mg kg ⁻¹)	
	Control	Seed Treated with Imidacloprid
Guttation Fluid	BDL	BDL
Pollen	BDL	BDL
Soil	BDL	BDL

BDL= Below Detectable Level; Below Detectable Level i.e. 0.01 ppm

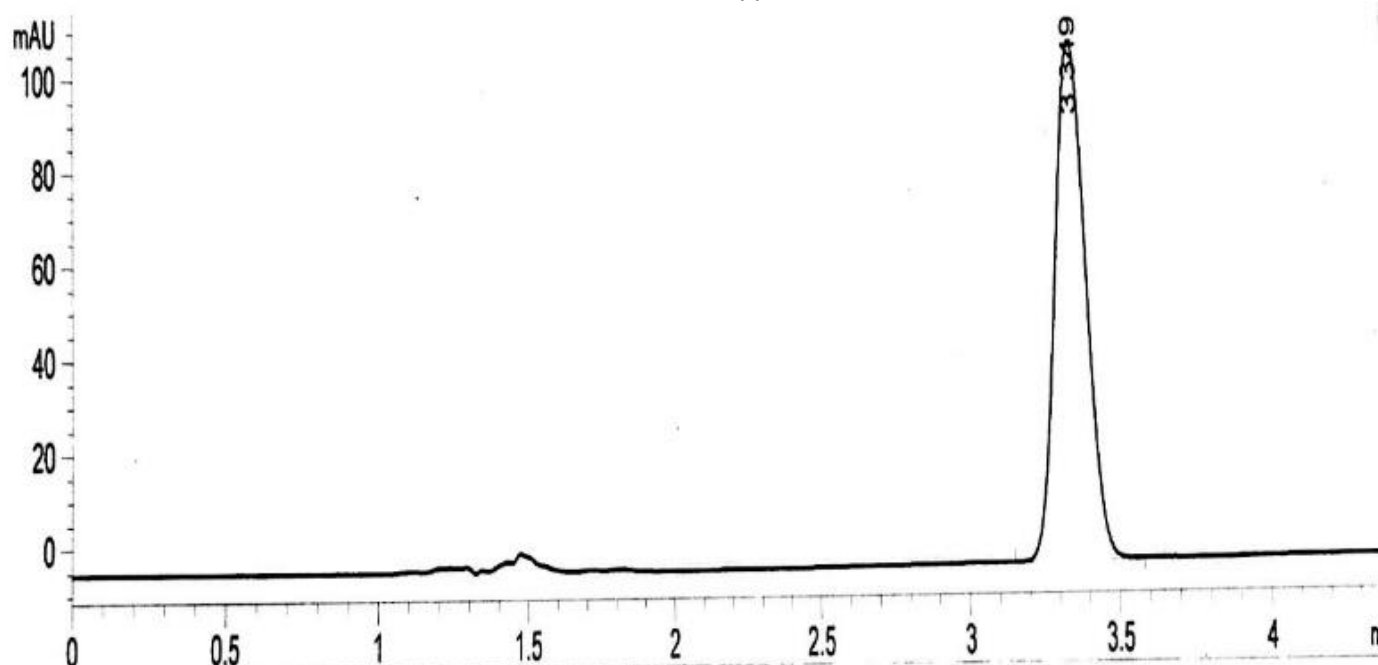


Figure 1. HPLC standard SCAN chromatogram showing the retention time of imidacloprid

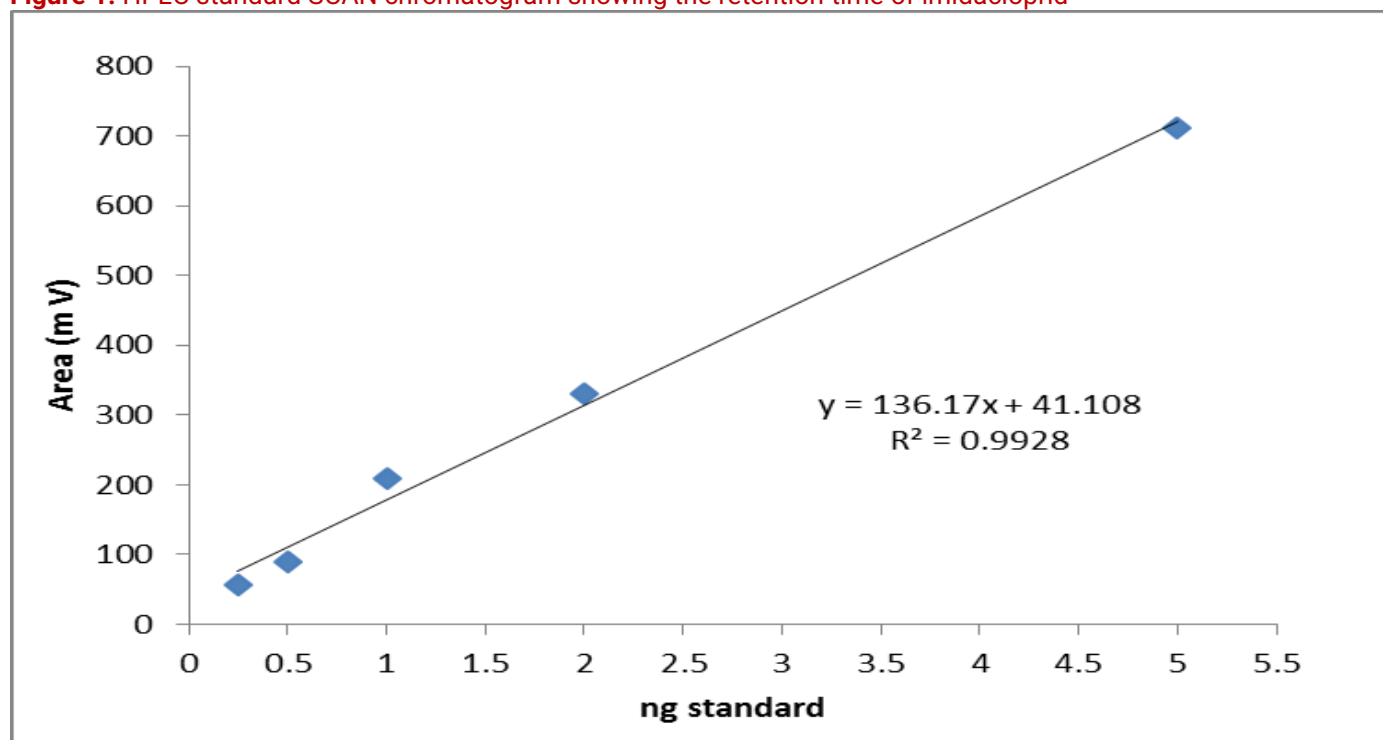


Figure 2. Standard curve of imidacloprid

Conclusion

An analytical procedure has been optimized in HPLC for the rapid determination of residue of imidacloprid in guttation fluid, pollen and soil in cotton crop. The method of analysis was run for 5 minutes and shows adequate

sensitivity, selectivity and excellent repeatability and detection limits for the intended purpose. It was revealed from the study that residue of imidacloprid used as a seed dressing was found below detectable level in the

guttation fluid and pollen (collected after 50 % flowering) of the cotton crop.

Acknowledgements

The authors are thankful to Head, the Department of Entomology and Head Cotton Section, College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar (India) for providing all necessary facilities and input requirements and for the experimentation.

Declaration of interests

The authors have no conflict of interest to declare.

Data sharing

All relevant data are within the manuscript.

References

- Blacquiere T, Smagghe G, Cornelis AMVG & Mommaerts V. 2012. Neonicotinoids in bees: a review on concentrations, side effects and risk assessment. *Ecotoxicology*, 21: 973-992.
- Bonmatin JM, Moineau I, Charvet R, Fleche C, Colin ME & Bengsch ER. 2003. A LC/APCI-MS/MS Method for analysis of imidacloprid in soils, in plants and in pollens. *Analytical Chemistry*, 75 (9): 2027-2033.
- Choudhary A & Sharma DC. 2008. Dynamics of pesticide residues in nectar and pollen of mustard (*Brassica juncea* (L.) Czern.) grown in Himachal Pradesh (India). *Environmental Monitoring and Assessment*, 144:143-150.
- Cui L, Qi H, Yang D, Yuan H & Rui C. 2016. Cycloxaprid: A novel cis -nitromethylene neonicotinoid insecticide to control imidacloprid resistance cotton aphid (*Aphis gossypii*). *Pesticide Biochemistry and Physiology*, 132: 96-101.
- Dhaliwal GS, Jindal V & Dhawan AK. 2010. Insect pest problems and crop losses: changing trends. *Indian Journal of Entomology*, 37: 1-7.
- Dikshit AK, Lal OP, Sinha SR & Srivastava YN. 2002. Safety evaluation, persistence and bioefficacy of Imidacloprid and beta cyfluthrin on okra. Part II. *Pestology*, 26(7): 30-37.
- Goulson D. 2013. An overview of the environmental risk posed by neonicotinoid insecticides. *Journal of Applied Ecology*, 50: 977-987.
- Helal A, Mukherjee I, Gopal M, Ametha OP, Patel BS & Somchoudhary AK. 2005. Multilocal field trials using Imidacloprid as seed treatment to propose its MRL in mustard (*Brassica campestris* Linn.) leaves. *Pestology*, 29 (7): 15-19.
- Kalyan RK, Saini DP, Meena BM, Pareek A, Naruka P, Verma S & Joshi S. 2017. Evaluation of new molecules against jassids and whiteflies of Bt cotton. *Journal of Entomology and Zoology Studies*, 5 (3): 236-240.
- Kamel A. 2012. Insecticide Residues in Pollen and Nectar of a Cucurbit Crop and Their Potential Exposure to Pollinators. *Journal of Agricultural and Food Chemistry*, 60(18): 4449-56.
- Kumar R, Kranthi KR, Monga D & Haldhar SM. 2009. Natural parasitization of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on cotton by *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) during 2008-09. *Journal of Biological Control*, 23 (4): 457-460.
- Kumar R, Kranthi S, Nitharwal M, Haldhar SM & Monga D. 2012a. Influence of pesticides and application methods on pest and predatory arthropods associated with cotton. *Phytoparasitica*, DOI 10.1007/s12600-012-0241-5.
- Kumar R, Monga D, Haldhar SM, Indoria AK, Chauhan R & Kranthi KR. 2012b. Impact evaluation of insecticide resistance management strategies in cotton under Technology Mini-Mission on Cotton (*Gossypium hirsutum*). *Indian Journal of Agricultural Sciences*, 82 (10): 852-857.
- Kumari B, Madan VK & Kathpal TS. 2008. Status of insecticide contamination of soil and water in Haryana, India. *Environmental Monitoring and Assessment*, 136: 239-244.
- Miles I. 1993. Imidacloprid: Pesticides leaching potential model. Report No. 105008.
- Ohno, Tomizawa M, Durkin KA, Naruse Y, Casida JE & Kagabu S. 2009. Molecular features of neonicotinoids pharmacophore variants interacting with the insect nicotinic receptor. *Chemical Research in Toxicology*, 22: 476-482.
- Pandiselvi P, Sathiyarayanan S & Ramesh A. 2010. Determination of spirotetramat and imidacloprid residues in cotton seed, lint, oil and soil by HPLC UV method and their dissipation in cotton plant. *Pesticide Research Journal*, 22 (2): 168-173.
- Reetz JE, Zühlke S, Spiteller M & Wallner K. 2011. Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? *Apidologie*, in press DOI: 10.1007/s13592-011-0049-1.
- Schenke D, Joachimsmeier I, Pistorius J & Heimbach U. 2010. Pesticides in guttation droplets following seed treatment – Preliminary results from greenhouse experiments. Poster SETAC Europe 20th Annual Meeting Seville, Spain, 23-24 May.
- Schenke D, Joachimsmeier I, Pistorius J & Heimbach U. 2011. Pesticides in guttation droplets following seed treatment- field studies. SETAC North America 32nd Annual Meeting, Boston, US 13-17 November.
- Schmuck R, Schoning R, Stork A & Schramel O. 2001. Risk posed to honeybees (*Aphis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Management Science*, 57: 225-238.
- Scholz K & Spiteller M. 1992. Influence of groundcover on the degradation of 14C- imidacloprid in soil. Brighton Crop protection conference – Pest and diseases. 883-888.
- Sur R & Stork A. 2003. Uptake, Translocation and metabolism of imidacloprid in plants. *Bulletin of Insectology*, 56 (1): 35-40.
- Tapparo A, Giorio C, Marzaro M, Marton D, Solda L & 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 & Casida JE. 2005. Neonicotinoid Insecticide Toxicology: Mechanisms of Selective Action. *Annual Review of Pharmacology and Toxicology*, 45: 247-268.
- Vennila S, Ramasundram P, Sheo R & Kairon MS. 2000. Cotton IPM and its current status. *Central Institute for cotton Research Technical Bulletin*, 8:1-13.
- Wallner KB. 2009. Guttation: Tropfen, die es in sich haben. *Deutsches Bienen- Journal*, 4:18-19.
- Zhang J, Percy RG & McCarty JCJ. 2014. Introgression genetics and breeding between upland and pima cotton: a review. *Euphytica*, 198: 1-12.