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A SURVEY BASED ON THE USE OF IMIDACLOPRID ON GREEN LEAFY VEGETABLES IN REMOTELY LOCATED SAGAR, INDIA

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ABSTRACT The low literacy level of the vegetable growers in the selected region of India is responsible for the use of highly toxic organophosphate pesticides on green leafy vegetables. Although in the Indian market imidacloprid (less toxic) is available but due to its high price and less effectiveness compared to that of organophosphates is not preferred to control pests on green leafy vegetables. The present research is a survey-based study designed to know the insecticide management practice among insecticide dealers, vegetable growers and comparison of obtained information with chromatographic results. The combination of survey and instrumental method (micellar liquid chromatographic technique) was utilized to determine whether the information collected from vegetable growers and insecticide dealers were similar and significantly correlated with analytical data or not. Among the selected 48 samples of green leafy vegetables, imidacloprid was detected only in 6 samples. The survey of the insecticide dealers and vegetable growers confirmed that the vegetable growers used toxic organophosphates pesticides instead of less toxic imidacloprid. Survey data collected from vegetable growers and pesticide dealers were also tested by chromatographic method and a significant correlation was found between them, which clearly demonstrates the minimum use of safe insecticide (imidacloprid). Imidacloprid insecticide was detected on green leafy vegetables after a preliminary survey of vegetable growers and pesticide dealers. Survey results revealed the use of potentially harmful pesticides other than imidacloprid which are banned from using in vegetables. Analytical results showed significantly less use of imidacloprid on green leafy vegetables.

KEYWORDS : Survey, Green leafy vegetables, Imidacloprid, Micellar liquid chromatograph

INTRODUCTION

In India with changing agricultural practices, the consumption of pesticides has increased many folds. The excessive use of these pesticides is quite evident due to their presence in vegetables, animal feeds, food products, packed food, and even in human breast milk (Chen et al., 2018; Chung & Chen, 2011; El- Sawi et al., 2012; Gill et al., 2020). Without proper removal of pesticide residues raw vegetable consumption may provide the direct entry of harmful chemicals into the human body which may pose severe health effects (Fallah et al., 2012; Yang et al., 2017). Pesticides show their toxic effects in two ways: acutely and chronically. Common acute effects are vomiting, nausea, convulsions, rashes, blisters, blindness, dizziness, diarrhea, death, etc. In contrast, chronic effects included cancer, neuro-diseases (acetylcholinesterase inhibition, Parkinson's disorder), endocrinal disruption, diabetes, leukaemia, asthma and so on (Kim et al., 2017).

In year 1994 imidacloprid (ICP) which belongs to the class of neonicotinoids (NN) was introduced as an alternative for traditionally used insecticides on green leafy vegetables (Kagabu, 2011). In the mode of action and chemical structure, ICP is similar to nicotine, however, it causes 700 folds lower mammalian toxicity than nicotine (Robin & Stork, 2003). Due to its low toxicity, it could become a good alternative for those insecticides that cause toxic effects on humans (Babazadeh et al., 2020), and the environment (G. Singh, 2001; Gupta & Prakash, 2009). Traditional insecticides have banned by Environmental Protection Agency (EPA) which includes monocrotophos, dichlorvos. However, both of these are still being practiced on vegetables (Gowri & Thangaraj, 2019; Jaiswal et al., 2019). Chlorpyrifos also banned by Environmental Protection Agency and Pesticide Action Network International due to its effect on nerve impulse transmission of the CNS of the insects.

Other insecticides like chlorpyrifos was also banned by Environmental Protection Agency (2018) and Pesticide Action Network International (2019) due to their various harmful effect including nerve impulse transmission of the insects.

In the past years, many survey-based studies were conducted using different chromatographic methods; TLC (Qamar, Afzal et al., 2017), HPLC-PDA (Harshit et al., 2017), GC-ECD (Farina et al., 2006), LC-MS/MS (Marschner et al., 2017) etc. The survey methods used were appropriate to know the sociodemographic and insecticide management practices among vegetable growers.

Instead of above mentioned chromatographic technique, micellar liquid chromatography (MLC) can be a method of choice due to its low cost, time-saving, and eco-friendly nature. MLC is nothing but HPLC technique where the composition of the mobile phase is completely different. It is a modified aqueous micellar mobile phase with a major part of water, surfactant (above critical micellar concentration), and low concentration of short-chain alcohols (i.e., propanol, butanol, pentanol) as an organic modifier. This change in the MLC method facilitates direct injection of the real samples after filtration into the chromatographic column (Bravo et al., 2020; Pahade). It saves time and money consumed in the stepwise extraction process for sample pre-treatment and reduces environmental pollution caused by bulk organic solvents in conventional chromatographic methods (Pawar et al., 2020).

The present research work is a survey-based study designed to know the insecticide management practice among insecticide dealers, vegetable growers and comparison of obtained information with chromatographic results. The direct interviews of vegetable growers and insecticide dealers were conducted to know insecticide management practice and its application pattern on green leafy vegetables in Sagar, which is situated in the underdeveloped, drought-prone area of Bundelkhand region of Madhya Pradesh in central India. Side by side, green leafy vegetables were analyzed using micellar liquid chromatography coupled with photodiode array detector (MLC-PDA) method to crosscheck the validity of data collected from the market. The combination of survey and instrumental method (MLC technique) was utilized to find out whether the information collected from vegetable growers and insecticide dealers were similar and significantly correlated with analytical data or not.

Experimental

Insecticide Survey And Data Collection

(a) Survey From Vegetable Growers

The present study was carried out in Sagar (M.P.), India. Insecticide application-related information on leafy vegetables was collected from vegetable growers. Forty (40) vegetable growers were interviewed and considered representative of local vegetable growers who come in the periphery of 20 km from the Sagar city center. The distance was defined because leafy vegetables have a short lifetime and it was challenging to transport them from long distances. The survey was conducted by direct interview method (questionnaire) in regional language. Information regarding types of leafy vegetables, the insecticide used, application rate per square meter (sqm), time of harvesting, frequency of harvesting, washing off vegetables etc. were collected.

(b) Survey From Insecticide Dealers

In the present work, the insecticide dealers of Sagar city were also interviewed. Ten (10) local pesticide dealers who cover the major part of the city in insecticide distribution were interviewed. Information was collected about insecticides which are currently in trend for application on green leafy vegetables, their daily selling, prices, effectiveness on target insects, instructions and recommendation for their customers about insecticide management etc. The dealer survey was conducted to check whether vegetable growers follow the instructions given by the dealer or not.

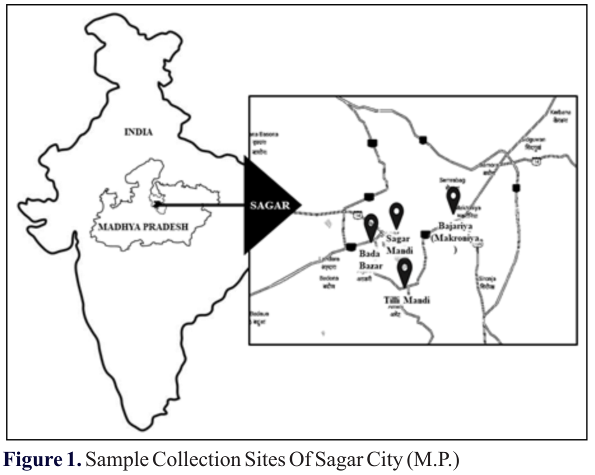

Figure 1. Sample Collection Sites Of Sagar City (M.P.)

(c) Sample Collection

The samples were collected during the winter season from four main vegetable markets of Sagar city i.e., Main mandi, Makronia bajariya, Bada bazar and Tilli road (**Figure 1**). These four vegetable markets are the hub where fresh vegetables arrive every day and from where retailers purchase the vegetables to be sold at the local level. Six types of leafy vegetable samples and two of each type were collected from the four main vegetable markets which are mentioned above. The vegetables included leaves of spinach (*Spinacia oleracea L.*), fenugreek *(Trigonella foenumgraecum L.)*, chickpea *(Cicer arietinum L.)*, onion *(Allium cepa L.)*, mustard *(Brassica juncea L.)* and coriander *(Coriandrum sativum L.)*. Therefore a total of 48 samples of the green leafy vegetables were collected. These are the main leafy vegetables consumed in this region during the winter season.

The sample collection strategy included the collection of two samples for each of the selected leafy vegetables. Around ½ kg sample from each category was bought and collected in a sterile plastic self-locking bag, which was stored in an ice chest box and transported to the laboratory. The samples were then prepared for further analysis in the laboratory.

Chemicals And Reagents

The standard imidacloprid (ICP) 98–99% pure was purchased from Sigma-Aldrich (Mumbai, India). The HPLC grade water was obtained from Indion Lab-Q Ultra water purification system, Ion Exchange (India) Ltd (Mumbai, India) for mobile phase and sample preparation. SDS to prepare micellar mobile phase and analytical grade sodium dihydrogen phosphate (99%) buffer to maintain the pH of micellar mobile phase were obtained from Himedia Laboratories Pvt. Ltd. (Mumbai, India). Solvents (1-propanol, 1-butanol, 1-pentanol, methanol) of HPLC grade, hydrochloric acid (≈37%) and sodium hydroxide (purity >99.0%) to adjust the pH of the mobile phase were obtained from Rankem, RFCL Limited (New Delhi, India). 0.45 µm nylon membrane filters from Micron Separation Inc. (Westboro, MA, USA) were used for filtration of all the solutions.

Sample Preparation

(a) Standard Samples

Standard stock solutions of 100 µg/mL were prepared in HPLC grade deionized water, stored in an amber glass volumetric flask and refrigerated at 4°C. The stock solution was serially diluted for MLC-PDA analysis. For the validation studies, the standard ICP solution was prepared in the 0.25-10 µg/mL range. The micellar mobile phase was prepared using 0.075 M SDS, buffered with 0.01 M sodium dihydrogen phosphate (NaH_2PO_4) at pH 7 (adjusted by 0.1 M NaCl). At last 2.5 % 1-propanol was added in the mobile phase as an organic modifier.

(b) Vegetable Samples

Leafy vegetables were chopped into small pieces and a 5 gm representative sample was crushed using mortar and pastel. After that the crushed sample was transferred to a 25 mL centrifuge tube and 5 mL water was added into it. The tube was centrifuged at 1500 rpm for 5 min. The supernatant was filtered using a 0.45 µm nylon membrane filter for chromatographic analysis (**Figure 2**).

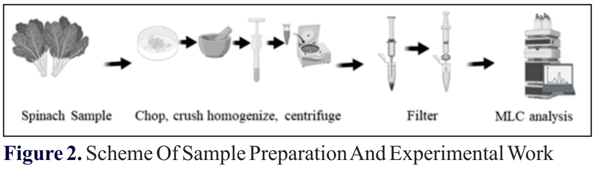

Figure 2. Scheme Of Sample Preparation And Experimental Work

Chromatographic Conditions And Software Processing

The MLC system used for the analysis was from Shimadzu Corporation (Shimadzu Prominence), Kyoto (Japan) having LC-20 AT isocratic pump, SIL-20AC auto-sampler and a photodiode array detector (SPD-M20A, 190-800nm) and a Shimadzu C_{18} analytical column (250 mm length × 4.6 mm internal diameter with 5 µm particle size). The Injection volume was 20 µL. The aqueous micellar mobile phase was running at 1 mL/min under isocratic mode. The instrument was operated and controlled through the software Shimadzu LC Solution software version 1.22 SP1. The tables, charts, chromatograms and diagrams were made using the software MS Excel, MS Powerpoint, Origin Pro 2018 and Biorender.

RESULTS AND DISCUSSION

Insecticide Supplier Survey

The present study started by conducting a survey on local insecticide dealers which clearly showed that conventional insecticides i.e. OPs are available at lower price (around ten times) in comparison to that of NN (ICP). Another observation noted during this investigation was that the concentration of ICP mentioned on the packing is lower than that of OP insecticide, making ICP less effective on insects than OP insecticides. The insecticide dealers also revealed that they always instruct vegetable growers about insecticide application time, intervals between subsequent application, concentration to be sprayed on particular leafy vegetables per square meter.

Table 1. Demographics And Insecticide Practice Management Among Vegetable Growers (n=30).

Age (%)	25-34	35-44	45-54	55-above
	56.66	16.66	20	6.68
Education level (%)	Illiterate	Primary	High school	Higher

	33.33	43.33	23	0.34
Gender (%)	Male	Female	Transgender	
	80	20	0	
Decision to apply insecticide (%)	Noticing insects and diseases	Friends recommendation	Calendar spray schedule	Noticing crop damage
	50	33.33	73.33	80
Quantity measurement method (%)	Bottle cap	Bottle	Weighing	Without any measurement
	70	10	6.66	13.34
Insecticide amount per acre (in 100 L of water) (%)	30 mL	60 mL	90 mL	120 mL
	10	16.66	60	13.34
Protective equipment during application (%)	No nasal and face mask	No hand gloves	No goggles	No protective clothing
	83.33	96.66	93.33	100
Method of insecticide application (%)	Mixing with seeds+ spraying	Spraying	Sprinkling	Dispersion
	66.66	13.34	16.66	3.34
Spraying intervals (%)	Weekly	15 days	20 days	25 days
	46.66	40	10	3.34

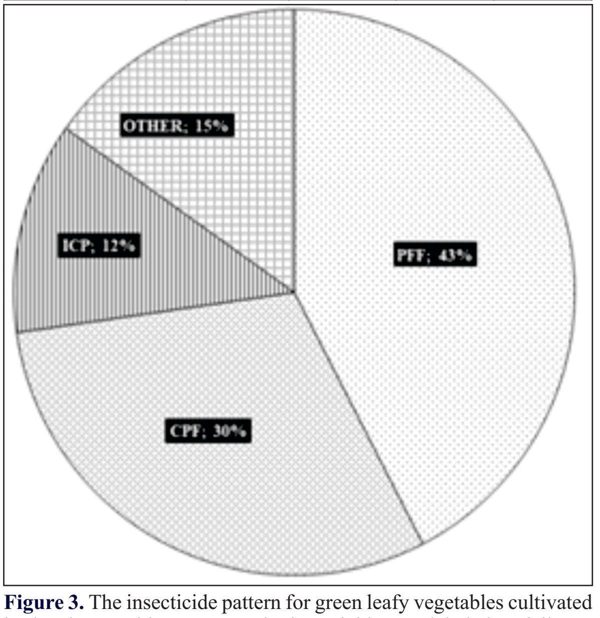

Figure 3. The insecticide pattern for green leafy vegetables cultivated by local vegetable growers. The insecticides are labeled as follows: CPF= Chlorpyrifos, PFF= Profenofos, ICP= Imidacloprid.

Background Of Vegetable Growers

Vegetable growers (total 40) who participated in the study were mostly male (80%), while less than 20% were female. The findings were similar to that reported by ***Belay T. Mengistie et al.*** in the vegetable farmers in Ethiopian Central Rift Valley (Mengistie et al., 2017). Age and education level were noted using well-defined ordinal scale categories. **Table 1** shows that the highest percentage of respondents (56%) belonged to 25-34 years age group, followed by 20% in 45-54 years and 16% in 35- 44 years age group and a small number i.e. 7% were above 55 years of age. Talking about the education level most of the vegetable growers i.e around 43% had attended primary or middle school, whereas 33 % of them were illiterate. It was also noted that only 0.34% have a higher education level.

General Insecticide Management Practice Carried Out By Vegetable Growers

As mentioned earlier, leafy vegetables are rich in nutrients and are considered good source of vitamins, minerals, fibers but are low in calories(Farina et al., 2016). Due to these properties leafy vegetables are quite healthy to be eaten either raw or in cooked form. They may be attacked by a variety of pests which may include flies, bugs, mites, worms and mollusks. Therefore to combat pests pesticides are commonly applied on green leafy vegetables. The vegetable growers of the selected city used different pesticides either in the form of liquids, granules or pellets. Insecticides most commonly used by the vegetable growers are Calcron (profenofos 50% EC), Dhanvan 20 (chlorpyrifos 20% EC), Polytril P440 (profenofos 40% + cypermethrin 4% EC), Nuvan (dichlorvos 76% EC), Confidor 17.8 (imidacloprid 17.80% SC). The insecticide application practice varied from person to person. Some of them relied on the suggestion of friends while others followed the instructions given by dealers. Maximum (70%) respondents were using bottle cap for measuring the amount of insecticide. However, only 6% used a weighing machine for measurement. Prevalent practices of insecticide quantities measurement are shown in **Table 2**. Around 46% of vegetable growers applied insecticide within a week of sowing whereas 66% used pesticides just at the time when germination started. Forty percent (40%) of the farmers were of the opinion that a cycle of 15 days was sufficient to control pests. However, due to changes in the weather pattern and the attack of particular insects on leafy vegetables the spraying interval may be reduced to 8-10 days instead of 15 days.

Common Insecticide And Pest Management Practices On Leafy Vegetable

In the survey, it was found that OP insecticides were the most frequently used insecticide on green leafy vegetables. This was around 73% of the total consumption of pesticides which included 43% CPF and 30% PFF whereas safer insecticide i.e. ICP was used by only 12% of the vegetable growers. Apart from common insecticides some of the other insectisides used by the vegetable growers are cypermethrin, indoxacarb, dimethoate, dichlorvos, and emamectin benzoate, which represented around 15% of the total pesticides used (**Figure 3**). During the survey it was also noted that spinach and coriander are cultivated throughout the year and are harvested 4-5 times with two insecticide treatments. Fenugreek, onion, chickpea and mustard are seasonal leafy vegetables that were cultivated only during the winter season. These vegetables were treated 2-6 times with an interval of 15 days (**Table 1**).

Table 2. Insecticide Management Practice By Insecticide Supplier (n=10)

S. No.	Insecticide	Instructions for farmers			
		Vegetable	When to apply after sowing?	How much/sqm	When to harvest after application
1	Chlorpyrifos 20% EC (Dhanvan 20, Vardan, Ramban, Chlorguard etc.)	Spinach, Chickpea, Mustard, Coriander, Onion, Fenugreek	15 days 50 days 15 days 20-25 days 20 days 15-20 days	0.25ml/50ml/sqm (irrigation for termite), 0.5ml/50ml/sqm (spray for insect control)	15 days
2	Imidacloprid (Confidor 17.8% SC, Imida 30.5% SC etc.)	Spinach, Chickpea, Mustard, Coriander, Onion, Fenugreek	15 days 50 days 15 days 20-25 days 20 days 15-20 days	0.2-0.25ml/50ml/sqm (irrigation for termite), 0.45-0.5ml/50ml/sqm (spray for insect control)	15 days
3	Profenofos 50% EC (Celcron, Paracron etc.)	Spinach, Chickpea, Mustard,	15 days 50 days 15 days	0.5-0.6ml/50ml/sqm (spray for insect and caterpillar control)	15 days

		Onion	20 days		
4	Profenofos 40% + Cypermethrin 4% EC (Polytril)	Spinach, Chickpea, Mustard, Onion	15 days 50 days 15 days 20 days	0.45-0.5ml/50ml/sqm (spray for insect and caterpillar control)	15 days
5	Dichlorvos 76% EC (Nuvan, Deroxa, Badal 76 etc.)	Spinach, Mustard	15 days 15 days	0.37-0.4ml/50ml/sqm (spray for insect and mite control)	15 days

Chromatographic Study For Validation

(a) Selection Of Chromatographic Conditions

The chromatographic analysis was performed according to the method developed by *J. E. Romero et al* for the determination of imidacloprid in wastewater with slight modification in the concentration of SDS (0.075M) and the wavelength of measurement (270 nm) (Mei-Liang et al., 2009). Imidacloprid is a moderately hydrophobic compound based on its octanol-water partition coefficient value of 0.57. Therefore the retention time of IPC on the C_{18} column with pure micellar eluents (without an organic modifier) was high (8.6 min). It was reduced by the addition of a small amount of short-chain alcohol (1-propanol). Finally, a mobile phase composed of 0.075 M SDS-2.5% 1-propanol, 0.01 M NaH_2PO_4 buffered to pH7 was used to carry out the chromatographic analysis of standard and real leafy vegetable samples. Using the selected mobile phase the retention time for ICP was 5.8 min.

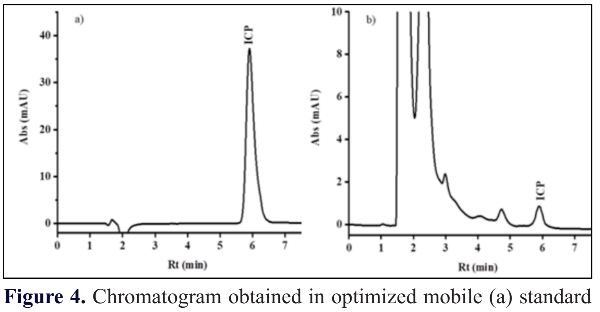

Figure 4. Chromatogram obtained in optimized mobile (a) standard ICP 10 µg/mL, (b) ICP detected in onion leaves at a concentration of 0.23 mg/kg.

(b) Linearity, Qualitative And Quantification Limit, And Recovery Of The Method

Method validation parameters; linearity, qualitative and quantification limit and recovery were calculated**.** Correlation coefficients 0.998 were obtained within the defined linearity range. Thus, the method showed good linearity for ICP. The LOD and LOQ were 0.01 µg/mL and 0.04 µg/mL, respectively demonstrating that method was sensitive to the analyte. Satisfactory recoveries (98 % to 104%) with 2.5% RSD were obtained from leafy vegetable spiked with 5 mg/kg of standard ICP.

(c) Evaluation Of Insecticide Residue In Leafy Vegetable

All 48 leafy vegetable samples collected from the selected sites were analyzed to detect the presence of imidacloprid (ICP) by MLC-PDA technique. The data obtained was used to crosscheck the vegetable growers statement and also to correlate it with the information collected from pesticide dealers. As shown in **Table 2** although the insecticide supplier of the selected city sold ICP, but it was detected only in 6% of analyzed samples (one onion and two mustard samples). The concentration of ICP detected was in the range of 1.33–2.34 mg/kg. The standard and real chromatogram of ICP are shown in **Figures 4a and 4b,** respectively. The results indicate that while ICP is non-toxic and safer among well-known insecticides available in the market but it was still not very popular among vegetable cultivators. The analytical results are identical to those obtained from insecticide suppliers and vegetable growers. Effort was not made to detect other insecticides in leafy vegetables because the present research work was aimed to know whether the vegetable growers were practicing the use of safe pesticides i.e. imidacloprid or not.

CONCLUSION

The present study evaluated pesticide management practices and their application pattern in green leafy vegetables grown around Sagar city (India). Due to the sensitive and delicate nature of green leafy vegetables towards insects and other pests, these vegetables cannot survive for a long time as other nonleafy vegetables. This increased susceptibility compels the vegetable growers to use highly toxic OP insecticides. The finding concluded that the main factors for using toxic insecticides were the low education level among vegetable growers, the high cost of ICP and effectiveness of easily available common insecticides in the market. This harmful insecticide practice was also confirmed by the MLC-PDA method, in which only 6 of samples were positive for ICP. It means that vegetable growers were using insecticides which are toxic for humans and environment. In the survey of pesticide suppliers, it was found that the vegetable growers visited them often in search of economical and highly effective insecticides and they completely overlooked the toxicity of these insecticides (around 10 times less expensive than ICP). According to the comprehensive study, there is an urgent need to educate vegetable growers to follow safety measures by conducting awareness programs and campaigns which should be organized by Universities in coordination with the respective governmental agency. The pesticide suppliers should also be educated and should suggest the vegetable growers to use pesticides that are less toxic than the routinely used insecticide.

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