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Harvest Time Residues of Imidacloprid and Thiamethoxam in Black Gram

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ABSTRACT

Imidacloprid and thiamethoxam insecticides have been important tools for controlling pests in black gram. The use of these pesticides on various crops has increased rapidly resulting the produce containing toxic residues from the application of these insecticides and in some cases the concentrations are above maximum residue limit. The prudent usage of synthetic insecticides resulted in secondary pest-outbreaks, resistance development, food, and ecosystem adulteration and so on which pose serious threat to human beings. However, food safety issues related to pesticide residues are important to consider with a food crop such as black gram which causes no harm to consumers and are permissible in domestic and international trade. Therefore, the objective of this study was to analyse harvest time residues of imidacloprid and thiamethoxam in black gram**.** The study was conducted during the summer season 2021 at Research Farm, Tirhut College of Agriculture, Dholi (Muzaffarpur)–Bihar. The crop (cultivar SML 613) was sown on February, 27, 2021. Insecticides were applied at 30 days after sowing which was followed by two successive sprays at 15 days intervals following the recommended dose of 25 g a.i. ha^{-1} for both the insecticides. Sample aliquots were analysed using HPLC (High Pressure Liquid Chromatography) equipped with Photo Diode–Array Detector (PDA). The sample taken at harvest with a pre–harvest interval (PHI) of 28 days did not reveal the residues of imidacloprid and thiamethoxam in black gram seeds at Limit of Quantification (LOQ) of 0.05 mg kg^{-1}.

KEYWORDS: Black gram, harvest time residues, imidacloprid, thiamethoxam

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Black gram, *Vigna mungo* (L.) Hepper is an important pulse crop grown in different part of India. Blackgram is native to Indian subcontinent (Nene, 2006). It constitutes an important source of cereals. In India, it is mostly grown in *kharif* season in an area of 54.39 lakh ha, total production of 35.32 lakh tonnes as against 0.168 lakh ha of area, total production of 0.100 lakh tonnes in Bihar (Anonymous, 2019). Blackgram contains carbohydrate (56.5 to 63.7%) and protein (24%) (Ali and Gupta, 2012) along with 3.2% of minerals, 154 mg calcium, 9.1 mg iron and 38 mg β-carotene per 100 g of split dal (Bakr et al., 2004). The low production of black gram in India, particularly in Bihar is due to avoidable losses by pests. Black gram and mung bean crops are predicted to lose over 30% of their yearly yields to insect pests each year (Rajawat et al., 2019), but the average annual avoidable loss from pests is between 15.62 and 30.96% (Duraimurugan and Tyagi, 2014). In India, 60 insect species were known to attack black gram at different stages of crop. (Lal and Ahmad, 2002). Among them, tobacco caterpillar (*Spodoptera litura*), blister beetle (*Mylabris pustulata*) (Boopathi et al., 2009), aphid (*Aphis craccivora*), *Amrasca biguttala biguttala* (Ishida) (Sundaryarajan and Chitra, 2014), grey weevil (*Myllocerus* spp.), Bihar hairy caterpillar (*Spilosoma obliqua*), gram caterpillar (*Helicoverpa armigera*), spotted pod borer (*Maruca vitrata*) are major foliage feeders and bugs like *Riptarsus pedestris* Fab. and *Clavigralla gibbosa* (Spinola) (Lal and Jat, 2014, Sundaryarajan and Chitra, 2014) feeds on pods whereas flower thrips (*Caliothrips indicus* Bagnall), whitefly (*Bemisia tabaci*) (Panduranga et al., 2011, Mishra and Mukherjee, 2015), jassid (*Empoasca* spp.) (Singh et al., 2010) and green leaf hopper (*Nephotettix* spp.) are sap feeders. In this study we mainly focus on thrips (*Caliothrips indicus* Bagnall (Dixit and Parihar, 2016, Bairwa et al., 2007), *Megalurothrips distalis* (Karny) (Mishra and Mukherjee, 2015)) and whitefly (*Bemisia tabaci* Gennadius)) (Panduranga et al., 2011, Mishra and Mukherjee, 2015, Taggar and Gill, 2012) are the most important pests during early stages of crop growth and also act as vectors of viral diseases (Swathi et al., 2019, Bhaskar Reddy et al., 2015) along with gram caterpillar (*Helicoverpa armigera* Hubner) which causes major damage during podding stage in subtropical India (Muthomi et al., 2008).

Over the last two decades, use of pesticides on various crops has increased rapidly (Thacker et al., 2005, Sharma, 2003). Therefore, the resulting produce contains toxic residues from the application of these insecticides and in some case the concentrations are above maximum residue limit (Dikshit et al., 2001). Hence a switch from these insecticides to new generation insecticides *viz.*, imidacloprid (Surulivelu et al., 2000) and thiamethoxam (Scarpellini and Nakamura, 1999) is needed which are required in significantly lower doses while maintaining the same toxicity level. Imidacloprid and thiamethoxam are effective against a variety of pests (Somasunder et al., 2016). The prudent usage of synthetic insecticides resulted in biodiversity losses, secondary pest-outbreaks, resistance development, pesticide-induced revitalization, and food and ecosystem adulteration which pose serious threat to human beings (Dhamaniya et al., 2005). There has been considerable concern with the pesticide residue accumulated in food items after their usage in pest management which causes no harm to consumers and are permissible in domestic and international trade. There is scarce literature available on residues of imidacloprid 17.8 SL and thiamethoxam 25 WG in black gram pods and seeds. Present study was undertaken to know about persistence behaviour of insecticides on black gram to ensure well–being of consumers under sub-topical conditions in Bihar, India.

2. MATERIALS AND METHODS

2.1. Field experiment

The sowing of the crop was done on 27th February, 2021. First spray of imidacloprid 17.8 SL @ 25 g a.i. ha^{-1} and thiamethoxam 25 WG @ 25g a.i. ha^{-1} was given on 2nd April, 2021 followed by 2nd spray on 17th April, 2021 with an interval of 15 days. Third spray was applied, 15 days after 2nd spray on 2nd May, 2021. The harvesting of black gram seeds was done on 30th May, 2021.

2.2. Sampling

After 28 days of the last pesticide application, harvesting and threshing of black gram seeds was done separately. From these harvested black gram seeds, 500 g samples were randomly collected. The collected samples were labelled and were brought to pesticide residue laboratory, Department of Entomology for further analysis.

2.3. Sample processing

A ground black gram sample (10 g) was transferred to a 50 ml polypropylene centrifugal tube and later kept overnight in refrigeration for homogenization. Samples were taken from the refrigerator and processed by following methodology given below:

2.3.1. Residue analysis of imidacloprid and thiamethoxam

"Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)" method with slight modification is used for processing of black gram samples for residue analysis.

Black gram (10 g)

↓

Dispensed 20 ml acetonitrile to centrifugal tube, cap well and shook

↓

Added sodium chloride (NaCl) 10 g and shook then centrifuged @ 2500 rpm

↓

16 ml acetonitrile transferred to a tube containing 10 g of anhydrous sodium sulphate and shook well ……... (1)

↓

Weighed "0.15±0.01 g PSA sorbent and 0.90±0.01 g anhydrous magnesium sulphate and add into 15 ml centrifuge tube for 6 ml extract ………..(2)

↓

6 ml aliquot taken from (1) to (2)

↓

Centrifuged @ 2500 rpm

↓

3 ml aliquot taken for estimating residues through HPLC

Schematic diagram of the method for analysis of imidacloprid and thiamethoxam in black gram

2.4. Estimation

The estimation of imidacloprid and thiamethoxam was done through HPLC (High Pressure Liquid Chromatography) equipped with Photo–Diode–Array Detector (PDA). The conditions to operate for imidacloprid and thiamethoxam were as follows:

Parameters	Imidacloprid and Thiamethoxam
Mobile phase	Acetonitrile: HPLC water (70:30)
Flow rate	0.3 ml min^{-1} (Imidacloprid); 0.2 ml min^{-1} (Thiamethoxam)
Wavelength	271 nm
Column temperature	40°C
Column	C18
Injected volume	20 µl
Detector	PDA

2.5. Calculations for the estimation of residue in test samples

The residues of imidacloprid and thiamethoxam in black gram were matched with the "retention time" of respective standards, whereas, estimated by "peak area". "Retention time" for imidacloprid and thiamethoxam was observed to be 2.95 and 4.40 min., correspondingly when injected under above mentioned conditions.

Quantification of residues (mg kg^{-1}) was calculated as:

Residue level mg kg^{-1}=(Pesticide standard injected (ng)/Peak height of standard injected)×(Peak height of the sample injected/Sample extract injected (µl)×(Final volume of the sample extract (ml)/weight of sample) ……………… (3)

2.6. Recovery experiments

Recovery experiments were carried out to check the efficiency of the analytical method used. Untreated black gram seeds (from control plots) were fortified with the imidacloprid and thiamethoxam standard with different levels of 0.5, 0.25, 0.05 mg kg^{-1}. These fortified samples were extracted, cleaned–up and analysed through method described earlier. The control samples from untreated plots and reagent blanks were also processed in the same way to find out the interferences, if any, due to the substrate and reagent, respectively

Percent Recovery=(Amount recovered/Amount added)×100……………………………………………………………(4)

3. RESULTS AND DISCUSSION

3.1. Limit of detectability of imidacloprid and thiamethoxam residue in black gram

In general, residues of both imidacloprid and thiamethoxam were determined by comparison of peak areas of the reference standards with that of the unknown or spiked samples run under identical working conditions of the instruments employed. The limit of detection (LOD) is the lowest amount of analyte detectable by an analytical instrument and is expressed in concentration units. The limit of quantification (LOQ) is the lowest, under the stated experimental conditions. It is also expressed in concentration units. The full–scale deflection was obtained with 5 ng of the standard of imidacloprid and thiamethoxam respectively. Samples of black gram were processed and terminal volume was composed to 3 ml The sample load of 20 µl for both imidacloprid and thiamethoxam was injected respectively, to observe the maximum load of samples can be analysed without any interference peak in the area relating to the compound estimated. The limit of quantification (LOQ) was found to be 0.05 mg kg^{-1} and limit of detection (LOD) was 0.017 mg kg^{-1} for both imidacloprid and thiamethoxam.

3.2. Recoveries of imidacloprid and thiamethoxam in black gram

The mean per cent recoveries of imidacloprid in black gram samples spiked of 0.05, 0.25 and 0.5 mg kg^{-1} range from 84.00 to 95.60% and found to be more than 80% (Table 1 and Figure 1). Whereas, mean per cent recoveries of thiamethoxam in black gram samples spiked with 0.05, 0.25 and 0.50 mg kg^{-1} levels ranged from 82.00 to 93.60% (Table 2 and Figure 2). In both the cases the amount recovered were greater than 80%, so the results obtained are expressed without application of any correction factor.

The quantitative determination of imidacloprid and thiamethoxam in black gram was validated as stated by

Table 1: Per cent recovery of imidacloprid from spiked samples of black gram				
Spiked level (mg kg^{-1})	Replicates		Per cent Mean Recovery±SD	RSDr
	Amount recovered	Per cent recovery		
0.05	0.046	92.00	87.30±0.04	4.76
	0.420	84.00		
	0.043	86.00		
0.25	0.231	92.40	89.70±0.02	3.13
	0.225	90.00		
	0.217	86.80		
0.5	0.478	95.60	93.20±0.02	2.38
	0.456	91.20		
	0.464	92.80		

SD: Standard deviation; RSDr: Relative Standard Deviation; Repeatability

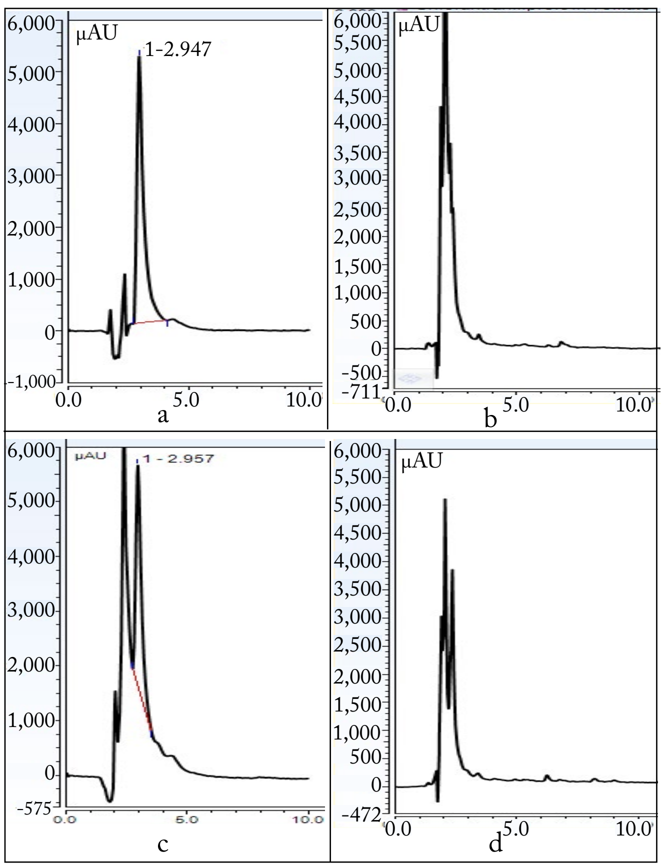

Figure 1: HPLC chromatograms: (A) imidacloprid standard (0.5 µg ml^{-1} (B) Control (C)black gram samples spiked with imidacloprid (0.5 µg ml^{-1}) (D) Treated sample

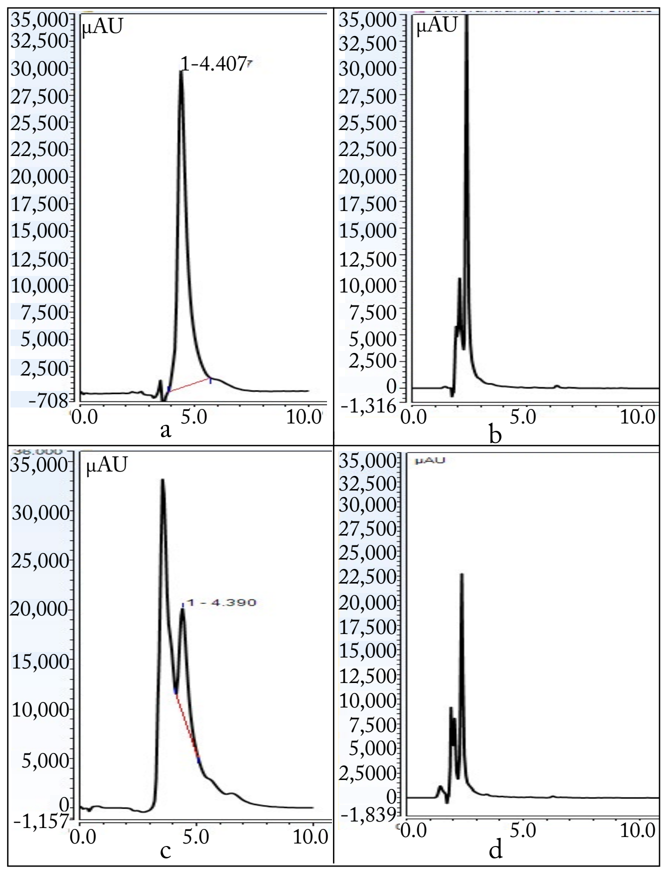

Figure 2: HPLC chromatograms: (A) thiamethoxam standard (0.5 µg ml^{-1} (B) Control (C)black gram samples spiked with thiamethoxam (0.5 µg ml^{-1}) (D) Treated sample

bio analytical method recommendations described in the SANCO guidelines. The calibration curves in relation to imidacloprid as well as thiamethoxam generate a linear relationship with different concentrations of 0.05, 0.1, 0.5, 1 and 2 µg ml^{-1} (Figure 3 and 4).

Determination of Repeatability (RSD_r) by spiking of imidacloprid and thiamethoxam through developed analysis method at different concentrations. The repeatability (RSD_r) for imidacloprid in black gram at 0.05, 0.25 and 0.5 mg kg^{-1}. level 4.76, 3.13 and 2.38%, respectively (Table 1). The repeatability (RSD_r) found for the thiamethoxam in black gram at 0.05, 0.25 and 0.5 mg kg^{-1} levels 4.65, 5.64

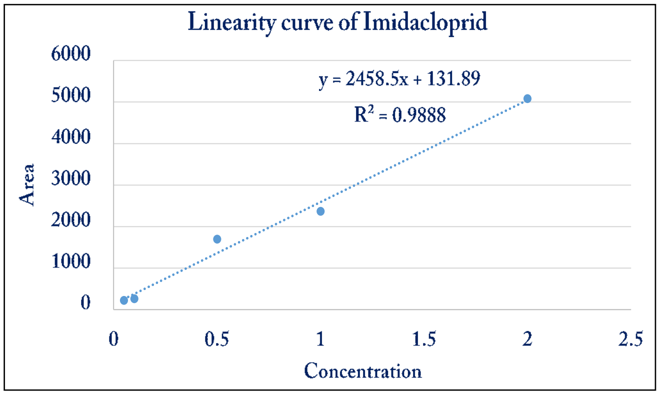

Figure 3: Linearity curve of imidacloprid standards

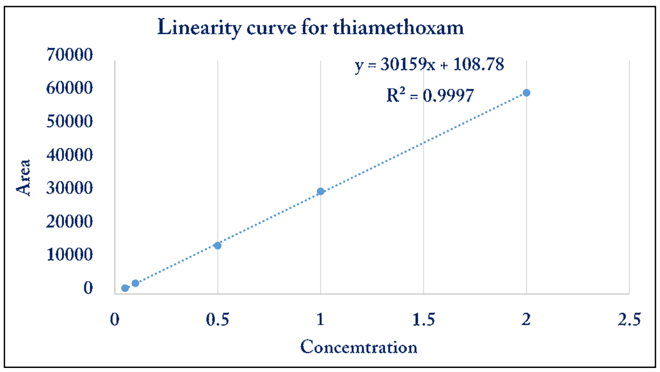

Figure 4: Linearity curve of thiamethoxam standards

and 2.69%, respectively. (Table 2).

The residues of imidacloprid and thiamethoxam in black gram seed samples collected 28 days after last treatment were below detected level. This is due to sufficient time gap between last treatment and harvest, such that the residues are dissipated and did not leave appreciable levels in black gram seed at limit of quantification (LOQ) of 0.05 mg kg^{-1} (Table 3). This was evidenced (Sivaveerapandian et al., 2002) from imidacloprid in okra stating that the residues were below detectable level even though the picking interval is minimum. This was in agreement with findings of Suganthi et al. (2018) for imidacloprid in chickpea green pods, for in stem and fruit samples of banana.

Table 2: Per cent recovery of thiamethoxam from spiked samples of black gram

Spiked level (mg kg^{-1})	Replicates: Amount recovered	Replicates: Per cent recovery	Per cent mean recovery±SD	RSDr
0.05	0.043	86.00	86.00±0.04	4.65
	0.045	90.00		
	0.041	82.00		
0.25	0.223	89.20	88.80±0.05	5.64
	0.209	83.60		
	0.234	93.60		
0.5	0.435	87.00	87.60±0.02	2.69
	0.451	90.20		
	0.438	85.60		

SD: Standard deviation; RSDr: Relative standard deviation; Repeatability

Table 3: Residue of imidacloprid @ 25 g a.i. ha^{-1} and thiamethoxam @ 25 g a.i. ha^{-1} in black gram seeds following its last application

Sample collection	Residue level (mg kg^{-1}): Replicates	Residue level (mg kg^{-1}): Mean±SD
At harvest (28 days after last spray)	<LOQ	<LOQ
	<LOQ	
	<LOQ	

LOQ: Limit of Quantification 0.05 mg kg^{-1}

4. CONCLUSION

The sample of black gram seeds were taken at harvest with a pre-harvest interval (PHI) of 28 days. Black gram seed samples did not reveal the residues of imidacloprid and thiamethoxam. Therefore, present study suggested a PHI of 28 days may be safe for consumption of black gram seeds following good agricultural practices (GAP).

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