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RESEARCH ARTICLE

Residue dynamics and risk assessment of Luna Experience® (fluopyram + tebuconazole) and chlorpyrifos on French beans (*Phaseolus vulgaris* L.)

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Abstract

The persistence of chlorpyrifos, fluopyram, and tebuconazole was estimated in green pods, matured seeds, and soil of French beans using dispersive QuEChERS. Three foliar applications each of chlorpyrifos and a combination fungicide fluopyram + tebuconazole (Luna experience, 400 SC) were applied at 600 and 125 + 125 as a standard dose and 1200 and 250 + 250 g a.i. ha^{-1} as a double dose, respectively, were applied at an interval of 10 days and treated pods were picked up at regular intervals. Dried mature seeds and soil were also monitored at harvest. The initial deposits of chlorpyrifos on bean pods were 3.083 and 6.017 mg kg^{-1} with a half-life of 1.86 and 2.29 days, at respective doses. Foliar application of a combi product Luna experience yielded 3.396 and 5.772 mg kg^{-1} residues of fluopyram and 3.613 and 5.887 mg kg^{-1} of tebuconazole in green pods at standard and double dose with almost same half-lives of 3.4 and 3.8–3.9 days. Residues declined below the limit of quantitation (LOQ) of 0.05 mg kg^{-1} in green beans after 15 and 25 days after the application of double dose of chlorpyrifos and Luna experience, respectively. However, the residues in dry bean seeds and soil reached below the LOQ of 0.05 mg kg^{-1} at the time of harvest. A pre-harvest interval of 5, 10, and 7 days has been proposed for chlorpyrifos, fluopyram, and tebuconazole, respectively, in beans. HQ < 1 and TMDI < MPI in all test chemicals. Hence, it was concluded that a waiting period of 5 days for chlorpyrifos and 7–10 days in Luna experience will be safer to consumers. This data generated will be useful for regulatory agency for fixing MRLs.

Keywords French beans · Chlorpyrifos · Fluopyram · Tebuconazole · Residues · Half-life · Risk assessment

Introduction

French bean (*Phaseolus vulgaris* L.) is one of the most commonly cultivated green vegetable grown throughout the world including India. China (16.20 MT) is number 1 in production of French beans or green beans in the world followed by Indonesia (0.87 MT) and India (0.62 MT) (FAOSTAT 2012). In India, it is grown on an area of about 1 lakh ha mainly in the states of Maharashtra, Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh Hills, Nilgiri (Tamil Nadu) and Palni (Kerala) hills, Chickmangalur (Karnataka), and Darjeeling hills (West Bengal) (Singh et al. 2016) with annual production of 1370.21 thousand MT in the years 2013–2014 (NHB, 2014). Himachal state situated in the North West corner of India with total area of 55,673 km^2 and is located between 48 30° 22′ 40″ to 33° 12′ 40″ N latitude and 75° 45′ 55″ to 79° 04′ 20″ E longitude region flanked by Jammu and Kashmir on its northwest, Uttaranchal on its east, Haryana in the south and Punjab in the west, in east it touches Tibet’s boundary. Cultivation of green beans in the state as off-season cash crop is undertaken on commercial scale having an ideal climate ranged between 15 and 30 °C on an alluvial soil with pH of 5.5–6.5. The crop is cultivated mainly by the small and marginal hill farmers of Himachal Pradesh, Jammu and Kashmir, and Uttrakhand over an area of about 26.75 thousand hectares. There is a huge demand for green vegetables in the plains as the production in hilly state (Sharma 2007) is optimal. The preference of Indian

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consumers mainly to have fresh and lush green vegetables and least for processed products provides a business opportunity to the growers living nearby the big cities or towns, generally referred as peri-urban areas to meet the requirement of consumers and earn higher profit. Many farmers try to maximize their income by selling directly to consumers, thus bypassing wholesalers and other middlemen.

The crop is attacked by a wide array of insect pests like bihar hairy caterpillar, blister beetle, bean bug, pod borer, and aphids including various pathogenic fungi like *Sclerotinia sclerotiorum*, *Uromyces appendiculatus*, *Fusarium solani* f. sp. *phaseoli*, *Rhizoctonia solani*, and *Pythium* sp. causal agents of white mold, bean rust root rot, respectively, also attack French beans. These pathogens damage stem, pods, roots, and other plant parts of the vegetable (Saxena 2009), and among these, most of the pests are controlled by non-approved insecticides due to the introduction of new guidelines issued by the Central Insecticide Board and Registration Committee (CIBRC) that no pesticide should be registered under 9(3) section of the Insecticide Act 1968 until its MRL (maximum residue limit) has been fixed by the Food Safety and Standards Authority of India (FSSAI).

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate], an organophosphorus broad spectrum insecticide applied on different vegetables to control various kinds of pests of agricultural importance. Chlorpyrifos 20 EC is registered for application on 15 different food commodities including beans against pod borer and black bug (CIBRC 2016). It is effective against both sucking and chewing insects of various vegetables (Prasad et al. 2009; Mahmood et al. 2014; Abdou et al. 2015; Khan et al. 2017).

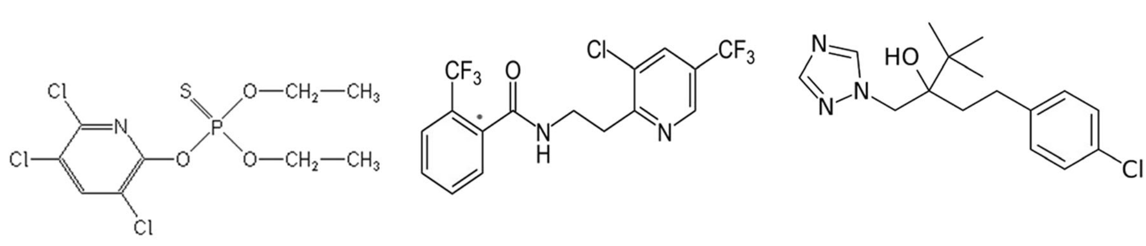
Another product evaluated for its dynamics on French bean was Luna Experience® introduced recently by Bayer CropScience Ltd. as a combi product of fluopyram and tebuconazole to control broad range of fungal diseases like powdery mildew and anthracnose in grapes (CIBRC 2016). Fluopyram is a new pyridylethylamide broad spectrum fungicide. It is highly efficient in controlling a wide variety of pathogens such as *Botrytis* spp., *Sclerotinia* spp., and *Monilia*, spp. and also against powdery mildews and leaf spot diseases in a broad range of crops (Zhang et al. 2014). Fluopyram affects the fungal growth at all the stages beginning from germination to sporulation. Its mode of action relies on the inhibition of succinate dehydrogenase (complex II) that is present in fungal mitochondrial respiratory chain. Tebuconazole ((RS)-1-p-chlorophenyl-4,4-dimethyl3-(1H-1,2,4-triazol-1- ylmethyl) pentan-3-ol)) is a broad spectrum fungicide belonging to triazole group (FAO 1997). It is used extensively worldwide and is found to be effective against fusarium basal rot and rust of garlic (Soares and Kurozowa 1998; Koike et al.2001), white rot of onion (Jaime et al. 2000), anthracnose of chillies (Saha et al. 2016), and many more. Chemical structures of chlorpyrifos, fluopyram, and tebuconazole are presented in Fig. 1.

Chlorpyrifos (Bhandari and Ujagir 2004; Raina and Raina 2008; Samriti et al. 2012; Jyot et al. 2013; Chandra et al. 2014) and fluopyram (Guan et al. 2011; Wei et al. 2016) dissipation behaviors have been studied in various vegetables. Tebuconazole residue behavior and risk assessment in combination with fluopyram in onion and chili (Patel et al. 2016; Saha et al. 2016) in combination with trifloxystrobin on chili (Sahoo et al. 2012), tomato (Singh and Singh 2014), onion (Mohapatra 2014), and gherkin (Mohapatra 2015) have been documented. Therefore, the present investigation was carried out to study the residue behavior and risk assessment of chlorpyrifos, as its pre-harvest interval (PHI) in green beans has not been documented on the CIBRC website. However, in combi product, fluopyram + tebuconazole persistence data in green beans was generated for registration and fixation of MRLs by the regulatory authority to ensure food safety of the consumers.

Material and methods

Experiment layout

The experiment was laid out in randomized block design (RBD) at the experimental farmat an elevation of about 1260 m above mean sea level, situated at 33.3° N latitude and 70.70° E longitude of the Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan (HP) during kharif season of



(a) (b) (c)

Fig. 1 Chemical structures: **a** chlorpyrifos, **b** fluopyram, **c** tebuconazole the year 2016. French bean (*Phaseolus vulgaris*) variety Green Wonder was raised in a $3 \times 2\text{-m}^2$ plot as per university standard package of practices (Anonymous 2016a). The experiment was conducted in a randomized block design (RBD) using three replicates for each treatment. Three foliar applications of Chlorguard 20EC at 7-day interval and Luna Experience 400 SC at an interval of 10 days were given at pod development stage with knapsack sprayer fitted with hollow cone nozzle. Chlorpyrifos was applied at standard dose of 600 g a.i./ha and double dose of 1200 g a.i./ha whereas the combi fungicide was sprayed at 125 + 125 g a.i./ha (standard dose) and 250 + 250 g a.i./ha (double the standard dose). The control plots were sprayed with water. During the experimental period of chlorpyrifos, average minimum and maximum temperatures were 17.08 and 28.45 °C, rainfall was 6.20 mm with average relative humidity of 73.50%, and the corresponding values during the study on the persistence of Luna Experience on beans were: average minimum and maximum temperatures 2.37 and 19.64 °C, rainfall 0.66 mm, and average relative humidity 57.68%.

Reagents

Analytical grade reagents viz., acetone, acetonitrile, n-hexane magnesium sulfate, sodium chloride, sodium sulfate, sodium citrate tribasic dehydrate, sodium hydrogencitrate sesquihydrate, and HPLC grade water were of Merck Specialties Pvt. Ltd. Primary secondary amines (PSA) were procured from M/S Agilent Technologies Pvt. Ltd. Worli, Mumbai. Formulated material Chlorguard 20 EC along certified reference material of chlorpyrifos (99.00%) was supplied by Gharda Chemicals and of a combi product Luna Experience 400 SC along with reference materials of fluopyram (99.40%) and its metabolite fluopyram benzamide (99.40%) and tebuconazole (99.10%) from Bayer CropScience Ltd.

Sample collection for persistence

In order to study the persistence, the green pod samples were drawn replication wise at an interval of 0 (2 h), 1, 3, 5, 7, 10, 15, 20, 25, and 30 days and dry pods at 45 and 50 days (harvest time) of last foliar application of chlorpyrifos and Luna Experience 400 SC, respectively. The soil samples from both the pesticide treatments were collected at harvest time only.

Extraction and dispersive solid phase cleanup

Green bean pods

The green pod samples were extracted and purified as per the modified QuEChERS method for vegetables (Sharma 2013). After last spray, bean pods (1 kg) were sampled and homogenized in Robot Coupe high volume homogenizer (Blixer, France). Samples (15 g) were homogenized with acetonitrile (30 ml) in high speed homogenizer (Heidolph, Germany) and sodium chloride (3 g) was added in the extraction to reduce the amount of polar interferences. Anhydrous sodium sulfate (9 g) was added to the representative aliquot to remove moisture contents. The extract was cleaned up with dispersive solid phase by using magnesium sulfate anhydrous (1.15 g) and PSA (0.4 g).

Matured bean seeds

Matured dried pods were harvested from the respective treatment and seeds were removed from the pods after sun drying. Approximately 100 g seeds were crushed/grinded in the blender. Then, 5 ml water was added to 10 g of representative sample in the centrifuge tube and soaked for 15 min, 30 ml of acetonitrile was added, and sample was processed as per the above mentioned QuEChERS method. The extracted matured bean seed samples were then subjected to dispersive solid phase cleanup.

Residues in samples of green bean pods and matured seeds were dissolved in 3 and 2 ml n-hexane, respectively; 1 μl of each was injected into GC/GC MS.

Bean field soil

Bean field soil (1 kg) was sampled and sieved. Soil was analyzed by QuEChERS technique, modified for analysis of soil (Asensio-Ramos et al. 2010). Soil sample (10 g) was extracted with 20 ml acetonitrile along with 4 g magnesium sulfate, 1 g of sodium chloride and to improve further recovery by the addition of 1 g of sodium citrate tribasic dihydrate, and 0.5 g of sodium hydrogencitrate sesquihydrate. After salting out, the representative aliquot is subjected to dispersive solid phase cleanup and analyzed by GC/GC MS.

Residue analysis

Chlorpyrifos residues were estimated by gas chromatograph (SHIMADZUGC 2010, Japan) equipped with FPD and capillary column (DB-5, 30 m long, 0.25 mm ID, and 0.25 μm film thickness). Temperature of injection port and detector was kept at 250 and 280 °C, respectively. Oven temperature was initially kept at 80 °C for 3 min, and then raised to 250 °C at the rate of 20 °C/min with a hold time of 20 min.

Fluopyram, fluopyram benzamide, and tebuconazole residues were estimated on GC MS. The cleaned extracts were analyzed on SHIMADZU GC 2010 equipped with DB-5 capillary column (30 m long, 0.25 mm ID, and 0.25 μm film thickness) coupled with MSD (GCMS-QP 2010 plus, Japan). The operating parameters for the column used were in a multiple ramp system, as follows: initial temperature was kept at 80 °C for 3 min, raised to 180 °C at 20 °C/min, and hold for 2 min followed by an increase to 280 °C at 5 °C with a hold time of 10 min. The injector port, ion source, and interface temperature were kept at 250, 200, and 280 °C, respectively. The flow rate of helium as a carrier gas was kept at 1.02 ml/min. Residues were estimated employing selected ion monitoring (SIM) mode. The selected ions for fluopyram were 173, 145, and 223 m/z, for its metabolite fluopyram benzamide were 173, 145, and 189 m/z, and the selected ions for tebuconazole were 125, 70, 250, and 83 m/z.

Method validation

To establish the linearity of the pesticides, correlation coefficient was worked out by running the standards of various concentrations viz., 0.05, 0.1, 0.25, 0.50, and 1.0 mg kg^{-1} and plotting the linearity curves. Accuracy is usually expressed as the recovery of known, added amounts of analyte (Francotte et al. 1996). Therefore, to further validate the method, recovery studies were done in green beans, mature bean seeds, and soil. Homogenized bean samples and sieved soil samples were spiked with chlorpyrifos, fluopyram, fluopyram benzamide (metabolite of fluopyram), and tebuconazole, at different levels 0.05, 0.10, 0.25, and 0.50 mg kg^{-1} (Fig. 2). Five replicates were taken at each fortification level. Accuracy and precision were worked out as percent recovery and RSD (SANTE 2017). The LOD is generally determined by considering a signal-to-noise ratio of 3 with reference to the back ground noise obtained from blank sample, whereas the LOQ was determined by considering a signal-to-noise ratio of 10 by using matrix matched standards. Therefore, the LOQ for various pesticides is 0.05 mg/kg and LOD is 0.017 mg/kg.

Data analysis of field study

The dissipation kinetics of chlorpyrifos, fluopyram, and tebuconazole at standard and double dose were calculated by plotting graphs between concentration and time. Three graphs were made and conclusion about rate of reaction was made as follows (Anonymous 2016b):

Concentration (C_0) Vs time (t) (best fit, if reaction is zero order); log C_0 Vs t (best fit, if reaction is first order); 1/ C_0 Vs t (best fit, if reaction is in second order).

Half-life (RL_{50}) values were calculated as described by Hoskins (1961) and Fantke and Juraske (2013):

For zero-order reaction:

$$t_{1/2} = \frac{A0}{2k_1} = \frac{A0}{2b}$$

For first-order reaction:

$$t_{1/2} = \frac{\log 2}{k_1} = \frac{0.301}{k_1} = \frac{0.301}{b}$$

For second-order reaction:

$$t_{1/2} = \frac{1}{k_1 A0} = \frac{1}{bA0}$$

where $t_{1/2}$: half-life value (RL_{50}) in days; A_0: initial deposit; k_1 (b): slope of regression equation of the log residues determined in mg kg^{-1} or ppm; (y) on the time elapsed in days (x).

Pre-harvest interval (PHI) was calculated as

$$T_{si} = \frac{\log k_2 - \log tol}{k_1} = \frac{\log k_2 - \log tol}{b}$$

where *Tsi*: time taken in days by the insecticide to reach tolerance limit; log k_2: log of initial deposit; log tol: log of proposed tolerance limit; k_1 (b): slope of regression equation.

There are no MRLs fixed for chlorpyrifos, fluopyram, and tebuconazole in green beans by FSSAI (Food Safety and Standards Authority, India). However, in other vegetables, MRL for chlorpyrifos is fixed as 0.2 mg kg^{-1} by FSSAI. Codex Alimentarius Committee has fixed MRL of 1 mg kg^{-1} for fluopyram in beans except broad beans and soybean (Codex 2016). For tebuconazole, the MRL have been fixed at 3 mg kg^{-1} in beans with pods by European Food Safety Authority (EFSA) (Brancato et al. 2017). On the basis of these MRLs, the PHIs have been estimated in green beans.

Calculations for dietary risk assessment

Dietary risk assessment was calculated using hazard quotient (HQ) as per Fu et al. (2016). Estimated daily intake (EDI) was calculated at the proposed pre-harvest interval and expressed in mg/kg/bw/day (Lozowicka et al. 2014).

$$\mathbf{EDI} = \frac{\text{Residue at proposed PHI} \times \text{daily consumption of beans}}{\text{Body weight}}$$

Daily consumption data of beans were taken from NSSO (2014) and hazard quotient was calculated as follows:

$$\mathbf{HQ} = \frac{\text{EDI}}{\text{ADI}}$$

where ADI is the acceptable daily intake.

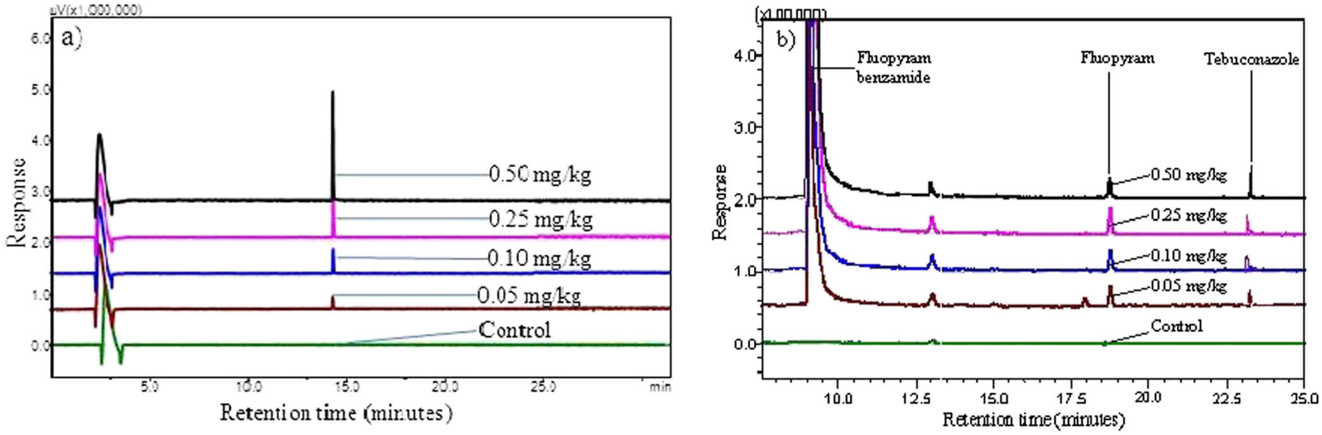


Fig. 2 Overlay chromatograph of French beans fortified with **a** chlorpyrifos, **b** fluopyram, its metabolite fluopyram benzamide, and tebuconazole, a part of combi product Luna Experience

Food consumption behavior of rural and urban community mainly depends upon agricultural production in India. Therefore, the HQ was determined to know the acceptable risk in rural and urban community. If the HQ worked out to be < 1, then the dietary risk considered to within the acceptable risk; however, if value observed > 1, it will represent the higher risk to consumer (Ludwicki et al. 2015; Malhat et al. 2014).

To evaluate overall dietary risk assessment of chlorpyrifos, fluopyram, and tebuconazole, analysis of the consumer's total diet was worked out using the theoretical maximum dietary intake (TMDI) for all the commodities in the Indian diet in a single day for which daily consumption data is available (NSSO 2014). TMDI was calculated as product of MRL and per capita consumption per day.

Comparison of TMDI values with maximum permissible intake (MPI) and the MRL assigned values of chlorpyrifos, fluopyram, and tebuconazole was made. MPI worked out by multiplying acceptable daily intake (ADI) with average body weight of human approx. 60 kg (ICMR 2010) and expressed as mg residue per person/day. If TMDI < MPI and MRL, then pesticides are considered to be within a safer limit.

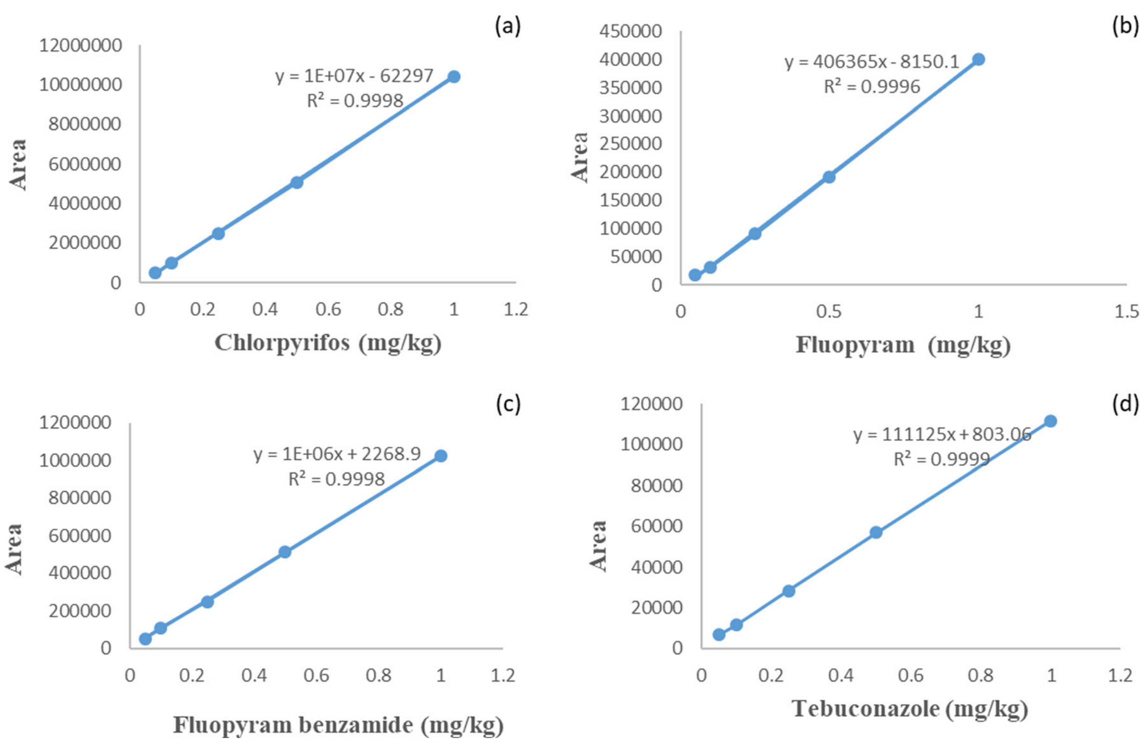


Fig. 3 Linearity of **a** chlorpyrifos, **b** fluopyram, **c** fluopyram benzamide, **d** tebuconazole

Table 1 Recoveries and percent RSD of chlorpyrifos, fluopyram, fluopyram benzamide, and tebuconazole from fortified green beans and mature bean seeds ($n = 5$)

Fortification level (mg kg^{-1})	Chlorpyrifos		Fluopyram		Fluopyram benzamide		Tebuconazole	
	% Recovery ± SD	% RSD	% Recovery ± SD	% RSD	% Recovery ± SD	% RSD	% Recovery ± SD	% RSD
0.05	96.00 ± 6.32 (95.00 ± 3.16)	6.588 (3.33)	92.00 ± 11.26 (97.00 ± 6.40)	12.293 (6.601)	104.00 ± 9.96 (101.20 ± 6.870)	9.689 (6.788)	106.00 ± 10.21 (104.40 ± 7.68)	9.612 (7.345)
0.10	97.00 ± 3.420 (97.00 ± 9.91)	3.526 (10.221)	87.00 ± 6.80 (99.20 ± 6.09)	7.857 (6.148)	108.00 ± 7.59 (107.80 ± 6.260)	7.046 (5.807)	92.00 ± 3.05 (103.20 ± 6.57)	3.329 (6.368)
0.25	95.20 ± 6.42 (96.40 ± 3.60)	6.746 (3.735)	100.40 ± 8.59 (96.00 ± 8.71)	8.532 (9.081)	96.00 ± 6.02 (96.40 ± 7.40)	6.280 (7.679)	92.00 ± 4.26 (102.80 ± 6.42)	4.629 (6.244)
0.50	96.40 ± 5.43 (97.00 ± 3.87)	5.633 (3.993)	109.00 ± 12.50 (102.60 ± 4.67)	11.849 (4.550)	98.80 ± 9.20 (97.60 ± 9.73)	9.324 (9.975)	91.80 ± 4.69 (92.40 ± 5.73)	5.107 (6.198)

Values in parentheses are per cent recoveries and per cent RSD in mature bean seeds

Results and discussions

Linearity

The linearity studies of chlorpyrifos, fluopyram along with its metabolite fluopyram benzamide, and tebuconazole at different levels were expressed as correlation coefficients (R^2). The R^2 value of chlorpyrifos was 0.9998 (Fig. 3a), and for fluopyram, its metabolite, and tebuconazole, the values ranged between 0.9996 and 0.9999 showing linear response in mass analyzer at 0.05, 0.10, 0.25, and 0.50 mg/kg (Fig. 3b–d). The values obtained were in accordance with the acceptable limit of > 0.99 as established by Hubner (2004).

Efficiency of method

Efficiency of analytical method was determined by conducting recovery experiments in green beans, bean seeds, and soil. In fortified green beans, the recoveries of chlorpyrifos ranged between 95.20 and 97.00% at spiking levels of 0.05, 0.10, 0.25, and 0.50 mg/kg using the aforesaid described method of extraction and cleanup, wherein the recoveries of fluopyram, fluopyram benzamide, and tebuconazole varied between 87.00 to 105.80, 96.00 to 108.00, and 91.60 to 106.00%, respectively (Table 1). Similarly, in mature bean seeds, the recovery of chlorpyrifos ranged between 97.00 and 108.00% at spiking levels of 0.05–0.50 mg/kg. The recoveries of fluopyram (87.00–109.00%), fluopyram benzamide (96.00–108.00%), and tebuconazole (91.60–108.00%) lie well within the acceptable range (Table 1). In soil, the recoveries of chlorpyrifos were between 93.60 and 101.60% and the recoveries of fluopyram, fluopyram benzamide, and tebuconazole ranged between 88.00 to 109.80, 100.00 to 110.00, and 97.00 to 106.00%, respectively, at spiking levels of 0.05, 0.10, 0.25, and 0.50 mg/kg (Table 2). All the recoveries were within the acceptable range of 70.00 to 120.00% and RSD is < 20 (SANTE 2017). Thus, based on the recovery studies and RSD, the method adopted was considered satisfactory for chlorpyrifos, fluopyram, and tebuconazole.

Persistence and residue studies

Beans

Chlorpyrifos Initial residue levels were 3.083 and 6.017 mg kg^{-1} in standard and double dose, respectively, after 2 h of the last application (Table 3). The residues reached to 0.065 and 0.073 mg kg^{-1} on 10th day and 15th day, respectively, in standard and double dose, showing degradation by 98 and 99% in this period. Residues further declined to below determination level (BDL), i.e., 0.05 mg kg^{-1} at 15th and 20th day, respectively, hence depicting almost 100% dissipation. In

Table 2 Recoveries and percent RSD of chlorpyrifos, fluopyram, fluopyram benzamide, and tebuconazole from fortified bean field soil ($n = 5$)

Fortification level (mg kg^{-1})	Chlorpyrifos		Fluopyram		Fluopyram benzamide		Tebuconazole	
	% Recovery ± SD	% RSD	% Recovery ± SD	% RSD	% Recovery ± SD	% RSD	% Recovery ± SD	% RSD
0.05	98.00 ± 6.54	6.650	96.00 ± 9.55	10.246	100.00 ± 10.00	10.00	106.00 ± 10.77	10.160
0.10	98.00 ± 4.62	4.690	107.00 ± 9.68	9.379	110.00 ± 8.20	7.485	97.00 ± 6.65	6.868
0.25	101.60 ± 10.25	10.073	88.00 ± 3.74	4.378	105.60 ± 9.96	9.454	99.20 ± 5.56	5.624
0.50	93.60 ± 4.12	4.402	105.96 ± 9.09	8.583	101.20 ± 10.32	10.217	103.20 ± 10.96	10.593

earlier studies on pigeon pea grains (Bhandari and Ujagir2004), brinjal (Jha et al. 2006), cauliflower (Raina and Raina 2008), okra (Samriti et al. 2012), and in green chili (Jyot et al. 2013) low initial residues of chlorpyrifos were observed (0.063–2.02 mg kg^{-1}) at different standard and double-dose level. However, Gogikar et al. (2017) reported more than two times the initial deposit of 14.73 mg kg^{-1} in curry leaves at 300 g a.i./h in comparison to our investigations. The variations in the initial residues of chlorpyrifos were attributed due to the difference in type of crop, dose of an insecticide undertaken in different agro climatic conditions.

Fluopyram and tebuconazole Residue dynamic studies of fluopyram in green beans revealed the initial deposits of 3.396 mg kg^{-1} from standard dose that dissipated to 0.053 mg kg^{-1} and at double dose, 5.772 mg kg^{-1} of initial deposits reached up to 0.054 mg kg^{-1} at intervals indicated in Table 3. The dissipation percentage varied between 34.37 and 99.00% for both the doses at respective intervals. The residues in green bean persisted up to 25th to 30th days. Fluopyram benzamide metabolite deposits were below the limit of determination in both the treatments. Likewise, fluopyram, tebuconazole also dissipated with same pattern indicating initial residue levels of 3.613 and 5.887 mg kg^{-1} in green beans. The residue degraded to 98.53 and 99.00% with the level of 0.053 and 0.055 mg kg^{-1} in standard and double dose, respectively, following the fluopyram pattern of persistence (Table 3). Patel et al. (2016) have observed longer persistence of a combi product fluopyram and tebuconazole in spring onion at 75 + 75 and 150 + 150 g a.i./ha and the residues dislodged below 55% on the 7th day. However, in our investigation, first-order kinetic revealed that 50% residues degraded on day 4. Singh and Singh (2014) studied the residues of tebuconazole in tomato as combi product with trifloxystrobin and observed that the tebuconazole residues persisted for 7 days with the half-life of less than 1 day indicating that the dissipation of tebuconazole at a faster rate in tomato than beans.

Table 3 Residues and persistence of chlorpyrifos, fluopyram, and tebuconazole in/on green bean pods

Days after application	Mean residues ± SD (mg kg^{-1}) $n = 3$					
	Chlorpyrifos		Fluopyram		Tebuconazole	
	Standard dose (600 g ai/ha)	Double dose (1200 g ai/ha)	Standard dose (125 g a.i./ha)	Double dose (250 g ai/ha)	Standard dose (125 g ai/ha)	Double dose (250 g ai/ha)
0	3.083 ± 0.109	6.017 ± 0.057	3.396 ± 0.247	5.772 ± 0.307	3.613 ± 0.494	5.887 ± 0.308
1	2.023 ± 0.095	4.113 ± 0.028	2.199 ± 0.027	3.788 ± 0.163	2.413 ± 0.102	4.004 ± 0.233
3	1.008 ± 0.035	2.090 ± 0.057	1.277 ± 0.031	2.248 ± 0.016	1.353 ± 0.131	2.411 ± 0.048
5	0.758 ± 0.004	1.460 ± 0.028	0.755 ± 0.010	1.367 ± 0.070	0.902 ± 0.017	1.502 ± 0.115
7	0.260 ± 0.016	0.705 ± 0.007	0.532 ± 0.032	0.736 ± 0.004	0.547 ± 0.050	0.926 ± 0.030
10	0.065 ± 0.003	0.190 ± 0.006	0.216 ± 0.016	0.492 ± 0.075	0.264 ± 0.026	0.541 ± 0.053
15	BDL	0.073 ± 0.003	0.113 ± 0.008	0.212 ± 0.015	0.134 ± 0.006	0.258 ± 0.022
20	–	–	0.053 ± 0.004	0.132 ± 0.010	0.053 ± 0.001	0.153 ± 0.014
25	–	–	BDL	0.054 ± 0.001	BDL	0.055 ± 0.004
30	–	–	–	BDL	–	BDL

BDL below limit of determination

Harvest time residues of chlorpyrifos, fluopyram, and tebuconazole in mature bean seeds and soil

Residues of chlorpyrifos, fluopyram, its benzamide metabolite, and tebuconazole in dry bean seeds as well as in bean cropped soil were below the LOQ level at harvest time (45 days in chlorpyrifos and 50 days in combi product fluopyram and tebuconazole after treatment). Parallel observations of Mohapatra (2014) in onion bulbs and soil at harvest (after 30 days) were recorded for tebuconazole residues.

Statistical analysis, half-life (RL_{50}), and pre-harvest interval

Three graphs of concentration and time were plotted to study the dissipation kinetics of chlorpyrifos, fluopyram, and tebuconazole at standard and double dose (Fig. 4). All the pesticides followed first order of kinetics as indicated by the higher R^2 values in the range of 0.9713–0.9866 in comparison to zero and second order of kinetic reactions. Therefore, chlorpyrifos, fluopyram, and tebuconazole showed progressive degradation pattern in green beans irrespective of the doses. Half-life calculated from dissipation curves of first-order kinetics was 1.86 and 2.29 days for chlorpyrifos in French beans. Samriti et al. (2012) reported residue half-life values of 0.58 and 0.61 days at standard dose (250 g a.i. ha^{-1}) and double dose (500 g a.i. ha^{-1}) for chlorpyrifos in ready-mix formulation in okra fruits. The half-lives in the present investigation for fluopyram and tebuconazole were 3.37 and 3.90 days, respectively, in standard and double dose (Table 4). However, Patel et al. (2016) observed half-life values of 8.85 and 9.12 days for fluopyram and 6.69 and 7.72 days for tebuconazole in spring onion at 75 + 75 g a.i./ha (standard dose) and 125 + 125 g a.i./ha (double dose). In present findings, differences in half-life values could be attributed due to variation in substrates, doses, and agro-climatic conditions. The results of residual dynamics experiment conducted by Guan et al. (2011) showed that the half-lives of fluopyram in cucumber were 3.7–4.3 days, respectively, which corroborate with the present investigation. On the basis of persistence data and MRLs fixed by the regulatory agencies, pre-harvest interval of 5, 10, and 7 days has been proposed for chlorpyrifos, fluopyram, and tebuconazole, respectively, in beans when applied at double dose (Table 4). Abdou et al. (2015) proposed a pre-harvest interval of more than 21 days for chlorpyrifos in green pods of *Vicia faba* bean having higher initial deposits (34.60 ppm) in contrast to the present studies. However, our investigations are in agreement with the observations of Patel et al. (2016) in onion and Saha et al. (2016) in chili for the same combi product.

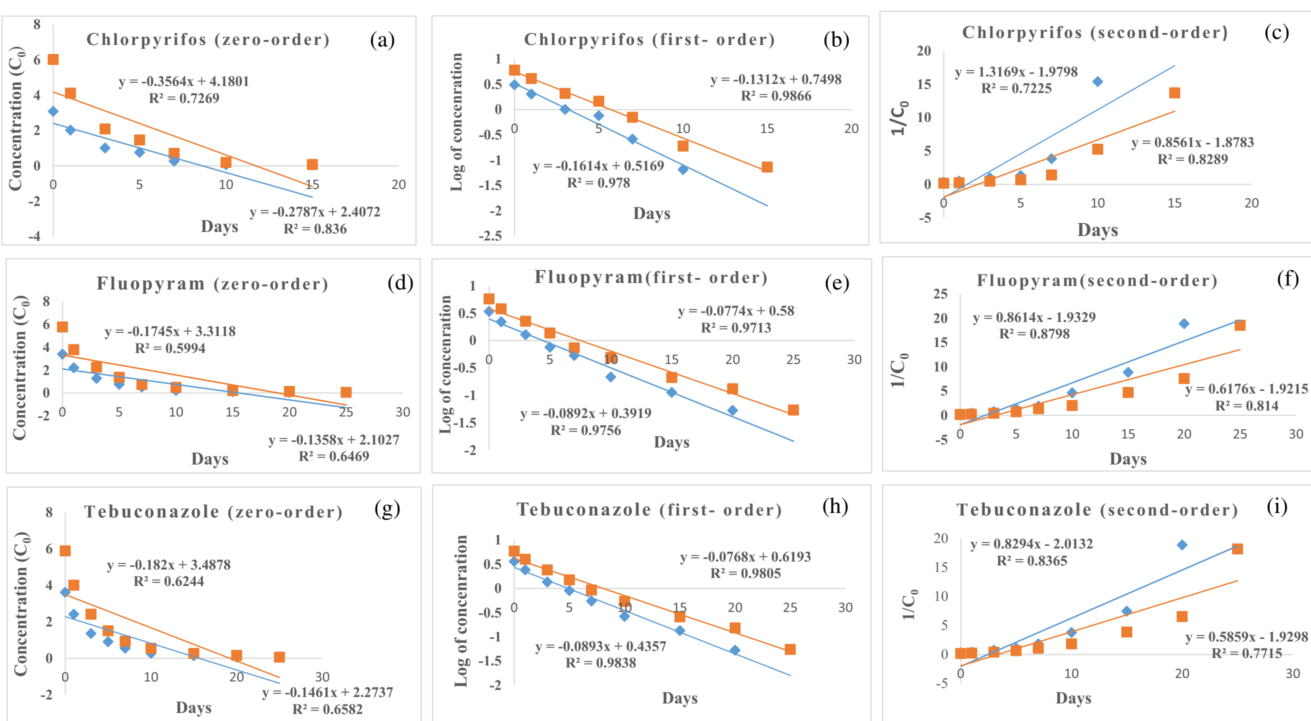


Fig. 4 Dissipation kinetics for pesticides in French beans at standard ◆ and double dose ■ **a** chlorpyrifos-zero-order kinetics, **b** chlorpyrifos-first-order kinetics, **c** chlorpyrifos-second-order kinetics, **d** fluopyram-zero-order kinetics, **e** fluopyram-first-order kinetics, **f** fluopyram-second-order kinetics, **g** tebuconazole-zero-order kinetics, **h** tebuconazole-first-order kinetics, **i** tebuconazole-second-order kinetics

Table 4 Dissipation kinetics: comparison of zero-order, first-order, and second-order kinetics of chlorpyrifos, fluopyram, and tebuconazole in French beans

Pesticide	Standard dose				Double dose			
	Regression equation	R^2	Half-Life (days)	PHI (days)	Regression equation	R^2	Half-life (days)	PHI (days)
Chlorpyrifos								
Zero order	y = − 0.2787x + 2.4072	0.836	5.53	–	y = − 0.3564x + 4.1801	0.7269	8.44	–
First order	y = − 0.1614x + 0.5169	*0.9780*	1.86	3.84	y = − 0.1312x + 0.7498	*0.9866*	2.29	4.55
Second order	y = − 1.3169x + 1.9798	0.7225	0.25	–	y = − 0.8561x + 1.8783	0.8289	0.19	–
Fluopyram								
Zero order	y = − 0.1358x + 2.1027	0.6469	12.50	–	y = − 0.1745x + 3.3118	0.5994	16.54	–
First order	y = − 0.0774x + 0.58	*0.9713*	3.37	5.96	y = − 0.0892x + 0.3919	*0.9756*	3.89	9.84
Second order	y = − 0.8614x + 1.9329	0.8798	0.36	–	y = − 0.6176x + 1.9215	0.814	0.28	–
Tebuconazole								
Zero order	y = − 0.1461x + 2.2737	0.6582	12.36	–	y = − 0.182x + 3.4878	0.6244	16.17	–
First order	y = − 0.0893x + 0.4357	*0.9838*	3.37	4.80	y = − 0.0768x + 0.6193	*0.9805*	3.92	6.98
Second order	y = − 0.8294x + − 2.0132	0.8365	0.33	–	y = − 0.5859x + 1.9298	0.7715	0.29	–

PHI pre-harvest interval

Values in italics show the best fit

Risk assessment

All pesticides always pose some level of risk. A 'risk assessment' is the process of evaluating the risks and benefits in using a pesticide. It often requires a complex combination of data to complete (NPIC, 2017). Risk assessment is crucial process of making decisions about the use of pesticides, both new and existing molecule. The presence of harmful pesticide residues is a cause of great concern among the consumers. The injudicious use of pesticides leads to harmful residues, which may constitute barriers not only to exporters but also to domestic consumers, when they exceed MRLs (Chahil et al. 2015). In India, FSSAI has not fixed the MRLs for chlorpyrifos, fluopyram, and tebuconazole on green beans; hence, theoretical risk assessment has been worked out by assessing hazard quotient and calculating TMDI values at proposed PHIs of 5, 10, and 7 days, respectively. HQ for all the three pesticides was found to be less than 1 at both the doses in both rural and urban diet (Table 5). Therefore, the present studies suggest that the application of chlorpyrifos and a combined product of fluopyram and tebuconazole on green beans are safer for humans at proposed PHIs. The values of TMDI for chlorpyrifos, fluopyram, and tebuconazole in all the food items are presented in the Table 6. Comparing the TMDI with MPI values of chlorpyrifos, fluopyram, and tebuconazole revealed that TMDI < MPI and MRL values for all the food items for which consumption data was available. Even the sum of TMDIs calculated for all the commodities was much lower than the MPI which shows

Table 5 Estimation of dietary risk assessment by calculating hazard quotient (HQ) in French beans (average body weight = 60 kg)

Pesticide	Dose	Residue (mg kg^{-1})	Daily consumption (kg) (rural)	Daily consumption (kg) (urban)	EDI (mg/kg bw/day) (rural)	EDI (mg/kg bw/day) (urban)	ADI (mg/kg)	HQ (rural)	HQ (urban)
Chlorpyrifos	Standard dose	0.758	0.0034	0.0046	0.000043	0.000058	0.01	0.0043	0.0058
	Double dose	1.460	0.0034	0.0046	0.000083	0.000112	0.01	0.0083	0.0112
Fluopyram	Standard dose	0.216	0.0034	0.0046	0.000012	0.000016	0.01	0.0012	0.0016
	Double dose	0.492	0.0034	0.0046	0.000028	0.000038	0.01	0.0028	0.0038
Tebuconazole	Standard dose	0.547	0.0034	0.0046	0.000031	0.000042	0.03	0.0010	0.0014
	Double dose	0.926	0.0034	0.0046	0.000052	0.000071	0.03	0.0017	0.0024

Table 6 Estimation of dietary risk assessment by theoretical maximum dietary intake (TMDI) in Indian food including different food commodities (average body weight = 60 kg)

Commodity			Chlorpyrifos				Fluopyram				Tebuconazole			
	Daily consumption (kg)		ADI (mg/kg)	MPI (mg residue/person/day)	MRL (mg/kg)	TMDI (mg/person/day)	ADI (mg/kg)	MPI (mg residue/person/day)	MRL (mg/kg)	TMDI (mg/person/day)	ADI (mg/kg)	MPI (mg residue/person/day)	MRL (mg/kg)	TMDI (mg/person/day)
Green beans	Rural	0.0034	0.01	0.6	0.2*	0.00068	0.01	0.6	1.00	0.0034	0.03	1.8	3.00**	0.0102
	Urban	0.0046				0.00092				0.0046				0.0138
Potato	Rural	0.0650	0.01	0.6	2.0	0.13000	0.01	0.6	0.03	0.00195	0.03	1.8	0.03	0.00195
	Urban	0.0540				0.1080				0.00162				0.00162
Onion	Rural	0.0280	0.01	0.6	0.2	0.0056	0.01	0.6	0.07	0.00196	0.03	1.8	0.03	0.00084
	Urban	0.0320				0.0064				0.00022				0.00096
Carrot	Rural	0.0260	0.01	0.6	0.1	0.0026	0.01	0.6	0.4	0.01040	0.03	1.8	0.4	0.0104
	Urban	0.0510				0.0051				0.02040				0.0204
Cauliflower	Rural	0.0090	0.01	0.6	0.05	0.00045	0.01	0.6	0.09	0.00081	0.03	1.8	0.05	0.00045
	Urban	0.0110				0.00055				0.00099				0.00055
Cabbage	Rural	0.0070	0.01	0.6	1.00	0.0070	0.01	0.6	0.15	0.00105	0.03	1.8	1.00	0.0070
	Urban	0.0090				0.0090				0.00135				0.0090
Banana	Rural	0.0100	0.01	0.6	2.00	0.0200	0.01	0.6	0.80	0.0080	0.03	1.8	1.50	0.0150
	Urban	0.0160				0.0320				0.0128				0.0240
Grapes	Rural	0.0012	0.01	0.6	0.50	0.0006	0.01	0.6	2.00	0.0024	0.03	1.8	6.00	0.0072
	Urban	0.0028				0.0014				0.0056				0.0168
Total TMDI	Rural					0.16693				0.02997				0.05304
	Urban					0.16337				0.04758				0.0871

All MRL values are Codex values except * indicates MRL value from FSSAI and ** indicates MRL value from EFSA that there is no risk involved if all the food items are consumed in a single diet.

Conclusion

The present investigations on the dissipation kinetics of chlorpyrifos, fluopyram, and tebuconazole showed that pesticides followed first-order kinetics at standard and double doses. The residues of chlorpyrifos, fluopyram, and tebuconazole persisted in green beans till 15th to 25th days. In all the cases, HQ is less than 1 and TMDI values are <MPI. Therefore, chlorpyrifos and a combi product fluopyram and tebuconazole can be used in green beans safely following a PHI of 5–10 days. As the residues were BDL in matured seeds and cropped soil, it can be concluded that the pesticides under investigation does not pose any threat. In India, no established MRLs exist for chlorpyrifos, fluopyram, and tebuconazole in green bean. Hence, present residue dynamic data along with other studies can be used for MRL fixation.

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