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Degradation dynamics of novaluron in chickpea using QuEChERS technique

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Abstract
Results of a field experiment conducted during 2015-16 to study degradation of novaluron in chickpea are reported. Novaluron (10 EC) at 37.5 (T_1), 75 (T_2) and 150 (T_3) g a.i ha^{-1} at pod formation stage. Samples were drawn periodically at 0 (2h after spraying), 1, 3, 5, 7, 10, 15, harvest time and analyzed by GC-ECD. Novaluron dissipated following first order kinetics with half life 1.40-2.73 in green pods and 2.31-3.13 in soil. The average initial deposits of novaluron in green pods and soil were found to be 0.642, 1.705, 2.251 and 0.144, 0.186, 0.223 mg kg^{-1} at T_1, T_2 and T_3, respectively. In case of green pods and soil 91 and 77% dissipation was observed at University recommended dose within 5 days after application. Harvest time grain and soil were found to be BDL.

Keywords: dissipation, novaluron, half-life, chickpea

Introduction
Chickpea commonly known as 'gram' or chana is a very important pulse crop that grows as a seed of a plant named *Cicer arietinum* in the Leguminosae family. It is the world's third most important food legume which contains 25% proteins and 61.1% carbohydrates (Singh and Yadav, 2007) [13]. India is the largest chickpea producer as well as consumer in the world. Chickpea contributes the single largest share in India's export basket of pulses registering 97.1 and 85.64% share in the total pulses export during 2013-14 and 2014-15, respectively, (Commodity Profile for Pulses-January, 2016). In Haryana, total area under chickpea cultivation is 83.0 thousand ha and total production of 75.0 thousand tones with the average productivity of 904 kg/ha (Anonymous, 2014). However, high yield is limited by the insect pests attacking chickpea. The major insect pests *i.e.* termites (*Odontotermes obesus*), cutworms (*Agrotis ipsilon*, *A. segetum, A. spinifera and Mythimna separata)* appear during seedling stage, while *Helicoverpa armigera* appear in great number during vegetative growth and at pod formation stage of chickpea (Lal, 1996). A number of insecticides belonging to cyclodiene, organophosphate, carbamates and pyrethroid group have been recommended for their control as insects have the ability to develop resistance to frequently and extensively used insecticides, there is always a need to identify new and safer insecticides for effective control.
Novaluron [(±)-1-[3-chloro-4-(1,1,2-trifluoro-2-rifluorometh-oxyethoxy)-phenyl]-3-(2,6-difluoro-benzoyl)urea] (Rimon 10 EC) (Fig 1) is known to be a reduced risk insecticide belonging to the group of insect growth regulators (IGR) (Tomlin, 2000) [14] and are comparatively safer to beneficial insects as well as to the environment. This novel benzophenyl urea IGR controls beet armyworm (*Spodoptera exigua*) and greenhouse whitefly (*Trialeurodes vaporariorum*) by Ishaaya *et al.* (1998) [5]. It also affects American ballworm (*H. armigera*) a larvae attacking cotton crop by Murthy and Ram (2002) [10]. It affects the pest maximum in the larval stages, which actively synthesize chitin, thereby causing abnormal endocuticular deposition and abortive moulting by Das *et al.* (2007) [3]. Hence, this compound is coming up as an eco-friendly or green pest-controlling agent.
Several reports are available regarding the detailed studies on bioefficacy of this compound. However, the data on the persistence and degradation pattern of novaluron on chickpea are not available as its residues may persist in green pods in amounts of above maximum residue limit (MRL) and pose health hazards to consumers. Keeping this objective in view, field studies were undertaken in 2015-2016 to investigate the dissipation behaviour of novaluron on chickpea greenpods, soil and mature grain (harvest time).

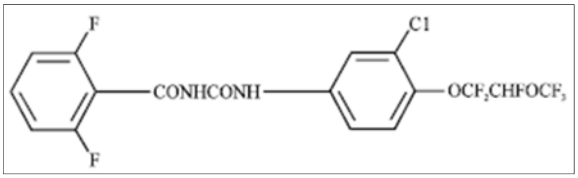

Fig 1: Chemical structure of novaluron

Materials and Methods

Chemicals and reagents

Reference standard of novaluron with a purity of 99.9% was obtained from Sigma Aldrich. n-hexane, acetone, and methanol were obtained from LiChrosolv, Germany. Primary secondary amine (PSA) and graphite carbon black (GCB) absorbents were purchased from Agilent Technologies. Anhydrous magnesium sulfate ($MgSO_4$) was procured from ACROS ORGANICS (USA).

The suitability of all the solvents and other chemicals was ensured by running reagent blanks before actual analysis. Standard stock solution of novaluron (400 mg/L) was prepared in methanol and stored at -4 ° C. Working standard solutions were acquired via appropriate dilution of the stock solutions with the same solvent ranging from 0.01 to 2 mg/L.

Field experiment

A field experiment using chickpea variety H 208 was laid out in a randomized block design (RBD) in 500m^2 plots according to the recommended agronomic practices in rabi 2015-2016 at the Research Farm of CCS Haryana Agricultural University, Hisar. A single spray of novaluron 10 EC at 37.5 (T_1), 75 (T_2) and 150 (T_3) g a.i ha^{-1} were given in separate plots at pod formation stage out of which T_1 is University recommended dose, T_2 and T_3 is double and triple dose, respectively by CIBRC. A plot of the same size but with no novaluron application was compared simultaneously, keeping as control, Samples were collected at random from treated plots at 0 (2 h after spraying), and 1, 3, 5, 7, 10 and 20 days after treatment. Immediately after picking, samples were brought to the laboratory and kept in freeze for further analysis.

Extraction and clean-up

Green pods

The samples were processed as per QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Sharma, 2013) for the determination of novaluron residues. The green pods were homogenized in a blender and 15 g of homogenate of each sample was taken in centrifuge tubes. Added, 30 ml acetonitrile (containing 1% acetic acid), and was homogenized at 14-15000 rpm for 2-3 min using low volume homogenizer. Then added 3g sodium chloride and mixed by shaking gently. The extracts were then centrifuged for 3 min at 2500-3000 rpm to separate organic layer. Took approximately 18 ml of upper organic layer in a test tube and added 9 g of Na_2SO_4 to remove moisture content. Transferred 11ml extract in a clean tube having 0.4g PSA and 1.15 g $MgSO_4$ and vortexed for 30 sec. The sample was again centrifuged for 5 min at 2500-3000 rpm. Then, 6 ml of supernatant was taken, evaporated to dryness, diluted with 3 ml of hexane, filtered through PTFE filter and transferred to a vial for GC analysis.

Harvest sample

Harvest time samples of pod covers and grains were ground in a Waring blender and dipped in hexane:acetone (4:1, v/v) by shaking for 1 hr on a mechanical shaker and the extract was cleaned with charcoal using hexane:acetone (4:1, v/v) as eluent (Kathpal and Dewan, 1976). Then, eluent was taken, evaporated to dryness and finally redissolved in 3 ml acetone for GC analysis.

Soil

Soil samples were extracted as per method of Kumari *et al.* (2008). To the well-ground, sieved and representative soil sample (20 g), added 0.5 ml ammonia solution and then left for half an hour. Ten grams of anhydrous sodium sulfate, 0.3 g Florisil and 0.3 g activated charcoal were added and mixed properly. The homogenized sample was packed compactly in a glass column (60 cm × 22 mm i.d.) in between two layers of anhydrous sodium sulfate. The column was eluted with 150 ml solution of hexane: acetone (1:1 *v/v)*. Eluate was concentrated to 5 ml on a rotary vacuum evaporator at 40°C followed by gas manifold evaporator to dryness. Final volume of the concentrated extract was reconstituted by adding n-hexane up to 2 ml and analyzed by GC.

Instrument for analysis

Residues were estimated on gas liquid chromatograph (GLC), Shimadzu, Model-GC 2010 equipped with ^{63}Ni electron capture detector (ECD) fitted with capillary column SPB-1 (30 m× 0.32 mm i.d. 0.25 µm film thickness (5% diphenyl + 95% dimethyl polysilioxane). N_2 was used as carrier gas at a linear gas velocity of 2 mL min^{-1} through column and make up flow at 60 mL min^{-1}. The injection port, 300°C, the oven temperature, 290°C and the detector temperature, 310°C was used for estimation. Retention time for novaluron was 2.79 min (Fig 2).

Calculation

Persistence data was fitted into first-order dissipation kinetics

$C_t = Coe^{-kt}$

Where C_t represents the concentration of the pesticide residue at the time of t, C_0 represent the initial deposits after application, and k is the constant rate of pesticide disappearance per day

In all cases, the first-order equation provided a satisfactory fit for the data ($r^2 > 0.9$) and the, the dissipation half-life periods were calculated based on this equation. Dissipation curve for chickpea and soil are shown in Fig 3 and 4.

Limit of detection and Limit of quantification

Quantification was accomplished by using a calibration curve prepared by serial dilutions (concentration 0.01 -2 ug ml^{-1}) of the stock solution. The minimum concentration with signal-to-noise ratio (S/N) of 3:1 was considered as the limit of detection and the S/N of 10:1 was fixed as the limit of quantification reliable linearity was achieved in the range of 0.05 -5 ug ml^{-1}. Thus limit of quantification (LOQ) was found to be 0.01 and Limit of detection (LOD) being 0.005 mg kg^{-1}.

Results and Discussion

Method validation

The analytical method was validated prior to the actual analysis. Recovery experiment was carried out at different spiking or fortification levels to establish the reliability and efficiency of extraction and cleanup procedures. Sample of green pod with seed and soil from control plots were spiked at level of 0.01, 0.05 and 0.10 mg kg^{-1}.These were extracted, cleanup and analyzed by the method already discussed. To ensure the specificity of the method towards the analyte, the

matrix effect was investigated by running sample blank. The mean percent recoveries ranged from 91.86-94.73 % in green pods and 85.96-89.46 % in soil, respectively (Table 1). Therefore, no correction factor was used and results have been presented as such.

Dissipation of novaluron

The dissipation data of novaluron for green pods and soil under field conditions are shown in Fig 3 & 4, respectively. The average initial deposits of novaluron in green pods were found to be 0.642, 1.705 and 2.251 mg kg^{-1}, following application @ 37.5 (T_1), 75.0 (T_2) and 150.0 (T_3) g.a.i.ha^{-1} on 0 day, respectively. The mean initial deposits were reduced to more than 35 per cent on one day. These residues were further reduced more than 90 per cent on 5th day. The residues reached below determination limit of 0.01 mg kg^{-1} on 7th day following application of novaluron @ 37.5 g.a.i.ha^{-1} whereas at 75 and 150 g.a.i.ha^{-1}, these residues reached below 0.01 mg kg^{-1} in 15 and 20 days, respectively, showing 100 per cent dissipation (Table 2).

In case of soil the average initial deposits at T_1, T_2 and T_3 dosages were 0.144, 0.186 and 0.223 mg kg^{-1}. At 37.5 g.a.i.ha^{-1} residues were reduced to BDL level on 7th day and at higher application @ 75.0 and 150.0 g.a.i.ha^{-1} residues persisted up to 7 and 10 days, respectively (Table 3). At harvest time, residues of novaluron in green pods and soil were below determination limit of 0.01 mg kg^{-1}. Novaluron dissipated in green pods and soil with half life 1.40 and 2.31 days at 37.5 g.a.i.ha^{-1}, 1.63 and 2.59 days at 75 g.a.i.ha^{-1} and at 2.73 and 3.13 days 150.0 g.a.i.ha^{-1}, respectively following first order kinetics.

Present findings in chick pea agree with the earlier data reported in chili and brinjal Das *et al.* (2007a) [4], where residues dissipated rapidly in chili and brinjal following first-order reaction kinetics @ 37.5 and 75.0 g.a.i.ha^{-1} application with half-lives of 1.80-1.95 days (for chili) and 1.8-2.08 days (for brinjal), respectively. Due to the lack of available data on dissipation study, it was not possible to perform much comparison with chickpea experiments. However, Malhat *et al.* (2014) did the terminal residue study and, the maximum residue of novaluron on tomato at the interval of three days after the last application was below 1 mg/kg and Saini *et al.* (2013) reported residue free chickpea grain, straw and soil at harvest time which corroborate the present findings.

Dissipation data showed that a gradual and continuous decrease of the novaluron residues in chickpea was observed. Slightly faster dissipation was observed in higher rate of application. Residues reached below MRL 0.1 mg kg^{-1} within 5 and 7 days at recommended dose (T_1), double the recommended dose (T_2), respectively. At higher dose (T_3) residues reached below MRL within 15 days and no phytotoxicity is observed.

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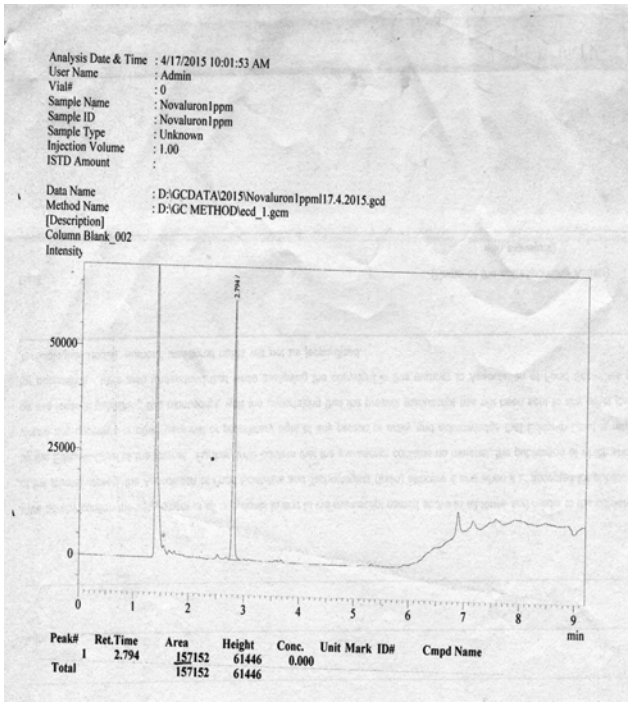

Fig 2: Chromatogram of Novaluron

Table 1: Recovery for novaluron in spiked chick pea samples at different levels

	Fortification Level (mg kg^{-1})	Recovery (%)	Average Recovery (%)±SD
Green pods	0.01	91.50	91.86±0.40
		91.80	
		92.30	
	0.05	93.20	92.66±0.46
		92.40	
		92.40	
	0.10	94.80	94.73±0.50
		94.20	
		95.20	
Soil	0.01	86.20	85.96±0.78
		85.10	
		86.60	
	0.05	88.20	87.63±0.55
		87.10	
		87.60	
	0.10	90.60	89.46±1.10
		88.40	
		89.40	

Table 2: Residues (mg kg^{-1}) and dissipation of novaluron in green pods

Application rate (g.a.i.ha^{-1})	Period after application	Average (mg kg^{-1}) (%)±SD	Dissipation (%)	Regression equation	R^2	Half life ($t_{1/2}$) days
37.5	0	0.642±0.14	-	2.803-0.215x	0.996	1.40
	1	0.402±0.02	37.38			
	3	0.131±0.03	79.59			
	5	0.056±0.02	91.27			
	7	BDL	-			
	10	-	-			
	15	-	-			
	20	-	-			
75.0	0	1.705±0.15	-	3.105-0.184x	0.974	1.63
	1	0.805±0.02	52.78			
	3	0.343±0.01	79.88			
	5	0.106±0.02	93.78			
	7	0.056±0.01	96.71			
	10	0.025±0.01	98.53			
	15	BDL	-			
	20	-				
150.0	0	2.251±0.07	-	3.148-0.11x	0.913	2.73
	1	1.212±0.04	46.15			
	3	0.742±0.14	67.03			
	5	0.212±0.01	90.58			
	7	0.144±0.01	93.60			
	10	0.122±0.07	94.58			
	15	0.045±0.01	98.00			
	20	BDL	-			

Table 3: Residues (mg kg^{-1}) and dissipation of novaluron in soil

Application rate (g.a.i.ha^{-1})	Period after application	Average (mg kg^{-1})	Dissipation (%)	Regression equation	R^2	Half life ($t_{1/2}$) days
37.5	0	0.144±0.03	-	2.192-0.130x	0.979	2.31
	1	0.120±0.00	16.66			
	3	0.071±0.01	50.69			
	5	0.032±0.00	77.77			
	7	BDL	-			
	10	-	-			
	15	-	-			
	20	-	-			
75.0	0	0.186±0.03	-	2.204-0.116x	0.955	2.59
	1	0.123±0.01	33.87			
	3	0.058±0.02	68.81			
	5	0.037±0.00	80.10			
	7	0.029±0.00	84.40			
	10	BDL	-			
	15	-	-			
	20	-	-			
150.0	0	0.223±0.04	-	2.363-0.096x	0.989	3.13
	1	0.180±0.01	19.28			
	3	0.131±0.04	41.25			
	5	0.081±0.04	63.67			
	7	0.042±0.01	81.16			
	10	0.026±0.00	88.34			
	15	BDL				
	20	-				

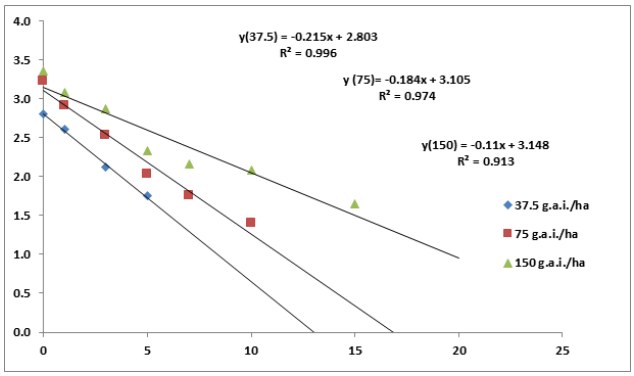

Fig 3: Dissipation of novaluron in green pods

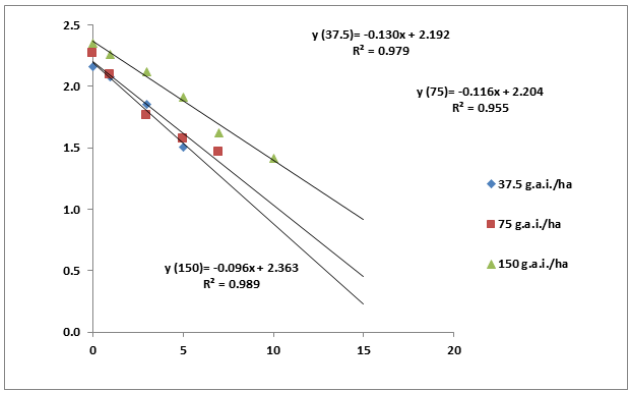

Fig 4: Dissipation of novaluron in soil

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