Original Research Article

Determination of Novaluron Residues in Soil under Chilli Pepper Ecosystem Using Liquid Chromatography Tandem Mass Spectrometry

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ARTICLE INFO

ABSTRACT

In this work, the persistence of novaluron (Rimon 10% EC) in soil was studied under the humid tropical chilli pepper ecosystem. Residues of novaluron in soil were determined using the modified QuEChERS method and liquid chromatography tandem mass spectrometry. The recovery values of the novaluron were found to be in the range of 88.4-105.2% with relative standard deviation ranging from 3.8 to 15.2% at 0.01, 0.05 and 0.1 µg/mL fortification levels in soil. The soil matrix influenced the ion enhancement in a tune of 22 and 28% at 0.05 and 0.1 mg/kg, respectively. The limit of detection was 0.005 mg/kg and limit of quantitation was 0.01 mg/kg. The initial deposit of the novaluron in soil at 37.5 and 75 g.a.i./ha dosed were 0.073 and 0.151 mg/kg, respectively, which reached below the limit of quantitation (0.01 mg/kg) after 5th and 7th days after the treatment indexing a half-life of 1.85 days for lower dose and 2.13 for higher dose.

GRAPHICAL ABSTRACT
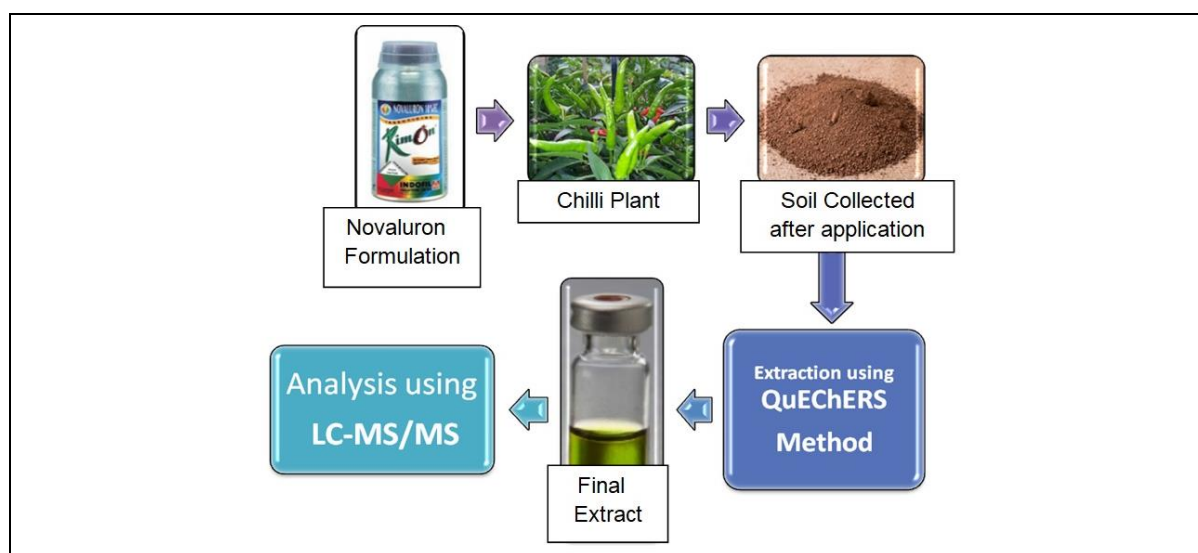
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Introduction

Agricultural chemicals have been accepted as important and prevalent means of achieving dependable and cost effective way of controlling pest in both the agriculture and public health. The continuous and over use of such chemicals (pesticides) may lead to environmental contamination [1]. It has been estimated that, less than 0.1% of the pesticide reaches the target pest, whereas the rest of the sprayed pesticides enters to the environment which may directly or indirectly contaminate soil, water, and air, wherever it can badly affect the non-targeted organism [2]. Novaluron, (±)-1-[3-chloro-4-(1,1,2-trifluro-2-trifluromethoxyethoxy)-phenyl]-3-(2,6-difluoro-benzoyl) urea (Figure 1) an insect growth regulator (IGR) [3] developed by Makhteshim-Agan Industries [4] is registered as insecticide [5] which troubling of normal growth and expansion of the insects. The IGR novaluron is used to protect chilli peppers against the fruit borers and tobacco caterpillars. The agrochemical is deemed to be an eco-friendly pest controlling agent even if it was very necessary to carry out the residue analysis in soil. Dissipation studies of novaluron in red loam soil are limited and therefore, such a research under humid conditions was conducted. The purpose of this study was to investigate the dissipation pattern of novaluron in soil under the field conditions.

Figure 1. Chemical structure of novaluron
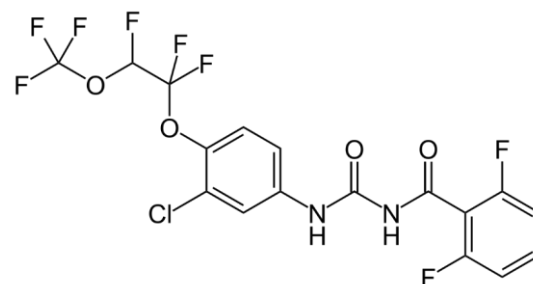
The compatibility of the solvents and other chemicals was ensured by running the reagent

Experimental

Materials and methods

The commercial formulation of novaluron (Rimon 10 EC) was procured from Indofil Industries (Mumbai, India) and the certified reference materials having purity >99.9% were purchased from the M/s Sigma Aldrich (St. Louis, MO, USA). Analytical grade chemicals including, sodium sulphate (Na_2SO_4), tri-sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$), anhydrous magnesium sulphate ($MgSO_4$), sodium chloride (NaCl), HPLC grade acetonitrile (CH_3CN) and ACS grade formic acid (HCOOH) were procured from the Merck (Mumbai, India). Disodium hydrogen citrate 1,5- hydrate ($C_6H_6Na_2O_7. 1,5 H_2O$) was also purchased from the Merck (Darmstadt, Germany). Chemicals such as NaCl, Na_2SO_4, and $MgSO_4$ were activated at 450 °C for 4 h before use. For the use of mobile phase in LC-MS/MS, water having a resistivity of 18.2 MΩ cm was prepared using the Elga Purelab water purification system (High Wycombe, UK) installed in the laboratory. The sorbents such as primary secondary amine (PSA), graphitized carbon black (GCB) and endcapped C18 were procured from M/s Agilent Technologies, USA. Optima LCMS grade ammonium formate ($HCOONH_4$) and polyvinylidene difluoride (PVDF) syringe filters (17 mm, 0.2 μm), were obtained from M/s thermo scientific (Rockwood, TN, USA).

blank samples before the actual analysis. A stock standard solution of concentration of

400 µg/mL of novaluron was prepared in methanol and stored at -20 °C. An intermediate standard stock solution of 10 µg/mL was prepared from the stock. The working standards of 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005 µg/mL were prepared by serially diluting with methanol from the 10 µg/mL solution, which is used for studying the linearity range, preparing matrix matched calibration standard and spiking for recovery studies. The calibration solutions were stored at 4 °C. Matrix matched calibration standards were prepared by adding the novaluron standard to the extracts of blank samples in the range of 0.005-1 µg/mL.

Instrumentation

The chromatographic separation of novaluron was achieved using the Acquity UPLC system (Waters, Milford, MA, USA) on the reverse phase Atlantis dC18 endcapped column (100 mm × 2.1 mm, 5 µm particle size) (Waters, Dublin, Ireland). The mobile phases consisted of [A] 0.1% HCOOH and 5 mM of CH_3COONH_4 in 10% MeOH in water; [B] 0.1% HCOOH and 5 mM CH_3COONH_4 in 10% water in MeOH. The gradient flow program was as follows: 20% B, 0-0.5 min; raised to 50% B, 0.5-1.0 min; then increased to 70% B, 1.0-2.0 min; raised to 90% B, 2.0-4.0 min; increased to 100% B, 4.0-6.0 min and decreased to the initial composition of 20% B at 6.0-8.0 min for re-equilibration. The sample and column compartment temperature were maintained at 5 °C and 40 °C, respectively. The injection

volume and flow rate of the method was 10 µL and 0.75 mL/min, respectively. The molecule, novaluron eluted after chromatographic separation was equipped with a triple quadrupole mass spectrometer, API 3200 (Sciex, Framingham, MA, USA). Ionization was achieved by the operating electrospray ionization (ESI) probe in the negative ion mode. The source dependent parameters were source temperature (550 °C); ion source gases (N_2), GS1 and GS2 (50 psi); ion spray voltage (-4500 V) and curtain gas (15 psi). For novaluron, a pair of scheduled multiple reaction monitoring (*S*-MRM) transitions were monitored and mentioned in Table 1.

DP-declustering potential, EP-entrance potential, CEP-collision cell entrance potential, CE-collision energy, CXP-collision cell exit potential, LC-MS/MS-liquid chromatography tandem mass spectrometry.

Method validation

The modified QuEChERS extraction method was validated in terms of linearity, specificity, limits of quantification (LOQ), limits of detection (LOD), accuracy and precision as per EU guidelines [6] prior to the real sample analysis. For the validation studies, a series of homogenized control samples of soil was fortified with novaluron at 0.01, 0.05, and 0.1 µg/mL in triplicates. The extraction and clean-up process for the soil is based on the QuEChERS method validated by Asensio-Ramos and co-workers [7] with slight modifications.
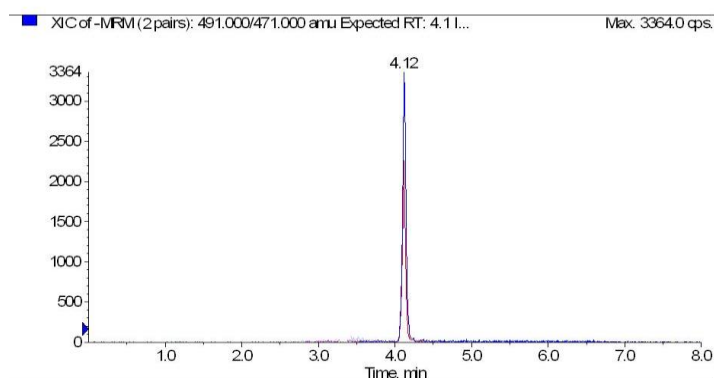
Table 1. Multiple reaction monitoring (MRM) parameters for novaluron in LC-MS/MS

Compound	Precursor ion *m/z*	Product ion *m/z*	DP	EP	CEP	CE	CXP	Quantitative/ Qualitative
Novaluron	491.0	471.0	-42	-5	-22	-20	-10	Quantitative
		156.0	-42	-10	-22	-21	-5	Qualitative

The red loam soil sample (10 g) was weighed in a 50 mL PTFE centrifuge tube to which 20 mL of acetonitrile is added. Add 2 g NaCl, 4 g MgSO$_4$, 0.75 g C$_6$H$_6$Na$_2$O$_7$. 1, 5 H$_2$O and 1.2 g C$_6$H$_5$Na$_3$O$_7$.2H$_2$O to the homogenized samples and immediately the tube is vigorously shaken for 1 min to prevent the coagulation of the sulfates. The extract was centrifuged at 10000 rpm at 8 °C for 8 min. The supernatant layer (16 mL) was transferred to a 50 mL centrifuge tube containing 5 g Na$_2$SO$_4$ which helps to remove the presence moisture, if any. Ten milli-liter of above supernatant layer was transferred to a 15 mL PTFE centrifuge tube and instantaneously added pre-weighed 1.5 g MgSO$_4$ and 0.25 g PSA. Vortexed for 2 minutes and is centrifuged at 10000 rpm at 8 °C for 8 min. The supernatant (4 mL) was evaporated to dryness with the help of a turbo Vap at 7.5 psi nitrogen flow and temperature of 40 °C. The residue was reconstituted to 2 mL using methanol and is filtered through 0.2 μm syringe filter for LC-MS/MS analysis. The coefficient of determination (R2) resulting from a five-point calibration curve indicates the acceptable linearity. Matrix effect study was carried out by comparing the area of matrix match standards with standard in pure solvent. The retention time of novaluron was 4.12 min (Figure 2).

Field experiment

The field experiments were conducted at farmer's field, Vellayani (8.4344° N, 76.9917° E), Kerala, India with one control and two treatment plots. The chilli peppers (variety-'Jwalamukhi'), was maintained as per the package of practices recommendations of Kerala Agricultural University [8]. The plots were in a randomized block design with three replications having plot size of 5×5 m each with a spacing of 60×60 cm. The experimental site was located in humid tropical climatic region, with 88 mm of average rain fall and 75% relative humidity. The chilli plants were cropped in red loam soil, of light texture with 45% sand, 28.5% clay, 5.55% silt, 0.65% organic carbon and the rest consist of coarse sand and gravel with the pH of 5.9. Novaluron 10% EC @ 37.5 and 75 g.a.i./ha was applied twice at an interval of 7 days starting from fruit setting with a hollow cone knapsack sprayer with spray volume of 500 L/ha.

Sampling, extraction, clean-up and analysis

The soil samples (red loam, 500 g) were randomly drawn from 0-10 cm depth using a soil tube auger from different spots in chilli field from each plot at 0 (after 2 h of spraying), 1, 3, 5, 7 and 10 days and was collected in an appropriate polyethylene bag. The samples were pooled, spread to dry in shade, ground and sieved through a 2 mm sieve (Mesh No. ASTM 10).

Figure 2. Chromatogram of Novaluron at 0.01 mg/kg level

The samples were analyzed as per the extraction method mentioned above. The residues were calculated using the Equation 1 [9].

Residues (mg/kg) = Concentration from the matrix calibration graph x Dilution factor   (Eq. 1)

Degradation of the residue data at different intervals was subjected to statistical analysis defined by Hoskin [10] to determine the half-life and pre-harvest interval. The half-life of the novaluron was calculated using the first-order rate equation, $C_t = C_o e^{-kt}$, where C_t is the concentration of novaluron at time t, C_o is the initial deposits after application, and k is rate constant of degradation. The half-life ($t_{1/2}$) is expressed as the time required to reach the half of initial concentration and was calculated from k value, $t_{1/2} = \ln(2)/k$.

Result and discussion

Efficiency evaluation

The analytical method used for analyzing the novaluron in soil indicated satisfactory results. The recoveries of the novaluron are in the range of 88.4-105.2% (Table 2), which was within the acceptable range of 70-120%. The precision of the analytical method expressed as RSD% (n=5) was between 3.8% and 15.2%. The linearity range of novaluron was from 0.01 to 1 mg/kg with $R^2 = 0.99$. The limit of detection (LOD) was 0.005 mg/kg and the limit of quantitation was 0.01 mg/kg. The effect of matrix on the ionization of novaluron was evaluated by comparing the area of neat standard with the matrix matched standard at 0.01 and 0.05 mg/kg, indicating that the novaluron in soil matrix have an ion enhancement effect occurred to a tune of 22 and 28%.

Persistence of novaluron in soil

The residue dissipation data of novaluron in soil was depicted in Table 3. The average initial deposits of the novaluron were 0.073 and 0.151 mg/kg at lower (37.5 g.a.i./ha) and higher (75 g.a.i./ha) doses, respectively. The residue values were dissipated by 47.94% and 52.98% on the next day at lower and higher doses. The data of dissipation indicated that within a day after the application of formulation of novaluron, the residues degraded to approximately 50%. The rest of the residues declined gradually and reached below the limit of quantitation after 5th and 7th day at 37.5 g.a.i./ha and 75 g.a.i./ha, respectively. The previous studies reported that the initial residues of novaluron in chickpea field soil [11] were 0.144 and 0.186 mg/kg at a dosage of 37.5 and 75 g.a.i./ha, respectively. Even though the dosages are same the slight difference in the residue values may be due to the difference in environmental conditions such as humidity, temperature, light, rain, plant variety *etc.,* [12]. Similarly, Anita and co-workers [13] reported that the initial residue values of novaluron in soil were 0.03 and 0.08 mg/kg at a dosage of 43.31 and 86.62 g.a.i./ha, respectively in tomato field, which is not corroboration with the findings of this study. This might due to the difference in crop, location of the experiment and also it was combi product study.

Table 2. Recovery of novaluron in soil

| Sl No.	Spiking level (mg/kg)	Average recovery (%)	RSD (%)
1	0.01	91.7 ± 1.05	7.4
2	0.05	88.4 ± 1.17	3.8
3	0.1	105.2 ± 1.21	15.2

Average recovery of five replicates; RSD-relative standard deviation

Table 3. Residues (mg/kg) of novaluron in soil

Days after spraying	Standard dose (37.5 g.a.i./ha)		Double dose (75 g.a.i./ha)	
	Mean residue ± SD mg/kg	Dissipation (%)	Mean residue ± SD mg/kg	Dissipation (%)
0 (2 h after spraying)	0.073 ± 0.011	--	0.151± 0.042	--
1	0.038 ± 0.007	47.94	0.071± 0.014	52.98
3	0.023 ± 0.008	68.49	0.040± 0.011	73.51
5	0.010 ± 0.004	86.30	0.031± 0.005	79.47
7	< LOQ	--	0.012± 0.003	92.05
10	< LOQ	--	< LOQ	--
Half-life ($t_{1/2}$)	1.85 days		2.13 days	
Regression equation	Y= - 0.1631 X+ 0.0838 R^2= 0.99		Y= -0.1416 X+ 0.1828 R^2= 0.99	

LOQ-Limit of quantitation, SD-Standard deviation

Figure 3. Semi-logarithmic graph showing the dissipation kinetics of novaluron in soil @ 37.5 and 75.0 g.a.i./ha
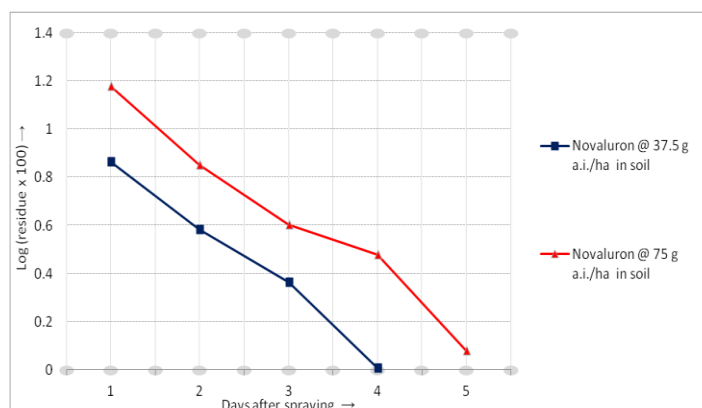
Conclusion

The insect growth regulator, novaluron is an eco-friendly pest controlling agent. It was very necessary to carry out the residue analysis of novaluron in soil. Dissipation studies of novaluron in red loam soil are limited and therefore, such a research under humid conditions was conducted. The purpose of this study was to examine the dissipation pattern of novaluron residues in soil under chilli eco-system. The modified QuEChERS methods were used for the extraction and dispersive solid phase clean up was adopted for the residue estimation of novaluron using liquid chromatography-tandem mass spectrometry. The average initial deposits of the novaluron were 0.073 and 0.151 mg/kg at lower (37.5 g.a.i./ha) and higher (75 g.a.i./ha) doses, respectively. The dissipation of novaluron in soil under chilli pepper eco-system followed first order kinetics with the half life of 1.85 and 2.13 days for lower and higher doses. The residues were reached below limit of quantitation (0.01mg/kg) of 7th and 10th day of lower and higher doses, respectively. Therefore, this study concluded that the use of novaluron formulation in chilli eco-system which cannot pose any harmful effect in the environment.

Acknowledgment

Disclosure statement

No potential conflict of interest was reported by the authors.

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