

Persistence and Degradation of Imidacloprid in Wheat Crop

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Abstract: Present study was conducted to investigate the levels and persistence of imidacloprid residues in wheat grains and straw of field crop samples grown from treated seed and foliar application. Objective of the study was to assess the best practices that may be used to produce safe grains and straw. Residual uptake of imidacloprid was measured after seed treatments at four dosage levels of seed. Each sample of 25g treated seed was sown in a separate 5ft² plot.

The absorption of imidacloprid residues was investigated by spraying the crops with 1ml and 5ml of 6 mgmL⁻¹ solution of 200SL Confidor (imidacloprid). The results helped in determining the maximum allowable limits of imidacloprid application (foliar or seed treatment) on wheat, which would prevent the residues from exceeding the MRL. The quantitative determination of imidacloprid suggested that the lowest seed treatment level (i.e. 0.015g/25g seed) may be used to produce a residues-free crop.

Keywords: Imidacloprid, wheat grains, wheat straw, persistence, seed treatment, foliar application.

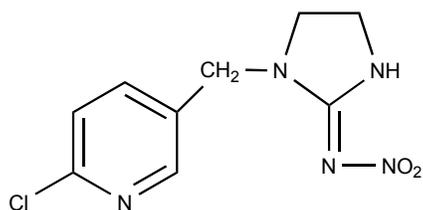
1. INTRODUCTION

Wheat plays a significant role in fulfilling the global food demand. Worldwide 225 million hectares is designated for sowing wheat crop [1]. Despite this huge cultivation area, wheat production doesn't meet the annual demand in developing countries including Pakistan due to comparatively low per hectare production of wheat. This may be due to various environmental factors, pests and diseases, such as attack of aphids on wheat crops which leads to transmission of barley yellow dwarf virus (BYDV) which in turn reduces the annual production by up to 3% [2]. Similarly, it has reported that BYDV is a widely distributed virus in wheat which may cause yield reduction up to 25% [3]. Therefore, controlling transmission of BYDV has gained a significant importance as it is an important factor for wheat production losses [4-7]. For this purpose, imidacloprid (IMI) has been reported as an effective tool to control BYDV on wheat [2,7-8]. IMI is a systemic chloronicotinoid insecticide with the ability to kill insects through ingestion or direct contact. Physiologically, it paralyzes nervous system of insects. It is generally used for controlling sucking insects and soil born insects therefore it is normally applied as seed and soil treatments [9,10]. Applications of this insecticide on

wheat crop in different parts of the world have shown to be beneficial to enhance yield and quality of wheat. Xue Xia *et al.* [11] reported the advantages (i.e. yield and quality) of using IMI over acetamiprid and water on wheat crop. It has also been reported that a quarter of imidacloprid application is equivalent to control the wheat aphids compared to with the application of omethoate [12]. Similarly, Simms *et al.* [13] investigated the use of IMI as seed dressing to control the wheat from slug damage. Despite the potential benefits of IMI, increasing application on wheat crop is also drawing attention in relation to food safety due to residual persistence of the insecticide in wheat grains. In this context, several insecticides persistence studies have been reported in wheat crop, including dimethoate [14], deltamethrin & cypermethrin [15], sulfosulfuron [16], and metasulfuron-methyl [17]. The persistence of the IMI has also been studied in different commodities. Sahoo *et al.* [18] estimated the residues of IMI in okra and reported the half-life between 0.85 and 0.96 days at 60g/ha and 120g/ha IMI dosage levels. Similarly, Donnarumma *et al.* [19] reported the translocation and residual degradation of IMI from seed to the maize plants. Many of researchers from all over the world, including Pakistan, have highlighted the agricultural importance of this insecticide, which makes it important to detect the residue levels of IMI in agricultural produces. This study has therefore been designed to investigate the minimum effective dosage level of IMI for seed treatment/ foliar application to

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produce grain and straw that are safe from the IMI residues perspective.



Molecular Formula:	C ₆ H ₁₀ ClN ₅ O ₂
Molecular Weight:	255.7 g mol ⁻¹
Melting Point:	143.8 °C
Water Solubility:	0.51g dm ³

2. MATERIAL AND METHOD

Uptake and absorption of IMI during the growth of wheat crop from treated seed and foliar applications standing crop were studied for consecutive three years (2010-11, 2011-12 and 2012-13). The experimental field crop was grown in the field of Cereal Crops Research Institute, Nowshera, Khyber Pakhtunkhwa, Pakistan.

2.1. Experimental Design

For this study, 625ft² field area was designated for growing experimental wheat crop. The total area was divided into 14 equal portions of 5ft². Between each two portions, 1ft distance was kept that helped to prevent the seepage/ transfer of insecticide form one sub-section to other. In the experimental filed, each two-adjacent 5ft² area was designated for each specific treatment whereas, two 5ft² areas were left unused. Each paired 5ft² experimental area was named as C, T₁, T₂, T₃, T₄, S₁ and S₂ and each of these part was distinguished by 'A' and 'B'. The field areas 'CA' and 'CB' were designated for control wheat grains (free of IMI treatment).

2.2. Treatment of Seed and Sowing

Commercially available 'IMI 25WP' (containing 25% active content) was used for seed treatments. Accurate mass of 25g of control wheat grains (free of IMI residues) were taken in eight separate beakers in two sets. The seeds in each four separate beakers were then treated accordingly with 50mL acetone containing 15, 30, 60 and 120mg of active content of IMI (each in duplicate). After complete evaporation of acetone and homogenization of IMI in wheat grains, these were taken to the field along with the two separate accurately weighted 25g of untreated wheat grains.

The untreated wheat grains were sown in the 'CA' and 'CB' field areas and the treated wheat grains i.e. 15mg/25g, 30mg/25g, 60mg/25g and 120mg/25g (in duplicate) were sown in the T₁, T₂, T₃ and T₄ experimental fields respectively. The selection of these treatment levels was based on the minimum and maximum recommended levels of seed treatment on wheat crop i.e. 50 to 175g per 100 kg of seed [20].

2.3. Foliar Application of Imidacloprid on the Crop

From the experimental fields, S₁ and S₂ field areas were selected for the foliar application of IMI. This application was performed before one month of harvesting. For this application, commercially available Confidor 200SL - 20% (w/v) was used and a solution was prepared that contained 6 mgmL⁻¹ of active IMI. 1mL and 5mL of this solution were diluted in 500mL of water in two different containers. These solutions were prepared in two sets. The solution prepared with 1mL of 6mg/mL was applied on both S₁A and S₁B field crops and the solution prepared with 5mL of 6mg/mL was used for S₂A and S₂B field crops. These solutions were filled in spraying chamber and were then sprayed on respective crop with the careful consideration of homogenized distribution of the insecticide on both grains and straw parts of the crop. Exactly the total volume of 500mL was sprayed on each 5ft² portion of crop to ensure the accurate amount of Confidor 200 SL was spread on experimental crop. The foliar application levels were selected following the recommended dosage i.e. 25-100g (a.i)/ ha [20].

2.4. Harvesting of Crop and Management of Sampling

At the time of harvesting, each stalk of wheat was cut down 6 inch below the lowest line of grain-stalk. About 500g of these samples from each field was separately collected. The samples of wheat grains and straw were randomly collected in sufficient quantity to ensure the samples were a true representative of each respective field. The samples collected from fields 'A' and 'B' were thoroughly mixed before transportation. The collected samples were sealed in separate opaque polyethylene bags and brought to the laboratory. In the first instance, the samples of grains and straw were separated. The grains were separately collected by removing them from shell.

2.5. Analysis of Wheat Grains and Straw Samples

Total quantities of each wheat grains and straw samples were individually mixed well and

homogenized. Before taking the sample into analyzing flasks, grains samples were ground and straw samples were cut into small pieces to increase their surface area and in turn for better penetration of extracting mixture. The extraction and cleanup were performed in accordance to method prescribed by Iqbal *et al.* (2012) [21]. Following the method, Four gram of each sample was taken into centrifuging tubes and shaken vigorously with 40ml mixture of acetone – methanol (1:1). The centrifugation was performed at 2500rpm for 3min and supernatant extracting mixture was filtered into a separating funnel through whatman filter papers. The step was repeating after addition of 35ml of extracting mixture. Dichloromethane (25ml) was added to the collected extract (75mL appx.) followed by 200ml of 2.5% (w/v) sodium sulfate. The extract was vigorously shaken and dichloromethane (DCM) along with imidacloprid residues was allowed to settle down at bottom of the separating funnel. The extract was then collected in a glass column containing 25g of anhydrous sodium sulfate supported by a glass wool at the bottom of column. Further 25ml of DCM was added to repeat the step twice. The collected extract was concentrated to approximately 2ml using rotary evaporator. The extract was thereafter transferred to 2nd glass column – filled with 13g of homogenized mixture of acidic aluminum and activated charcoal (12:1) in between the sodium sulfate layers. To clean up the column, the extract was driven over column using 160ml of DCM. The collected elute was then concentrated to dryness on rotary evaporator. Finally, the residues were dissolved in 2ml acetonitrile.

Samples thus processed were filtered through a millipore filter paper (pore size 0.45µm) before injection in a Shimadzu HPLC equipped with UV-VIS Detector SPD-10AV and two high pressure pumps LC-10AT. Beckman (Ireland) C18 column (5µm, 4.6mm x 15cm). For mobile phase, acetonitrile and water were placed in separate reservoirs linked with pump-A and pump-B respectively. Pumps were programmed to flow the mobile phase as a mixture of acetonitrile and water (8:2) with flow rate of 0.7ml/min. Total run time for each individual analysis was programmed for 10 min and after each run, mobile phase was allowed to flow for 5min with 1.0ml/min flow rate in order to remove undesired co-extractives that may interfere the next analysis.

3. RESULTS AND DISCUSSION

There are many published reports of wheat contamination with IMI and/or other pesticides in

different part of the world including Pakistan [22-27]. It is therefore of concern that the practice of improper foliar application of pesticides and/ or seed treatments on wheat in Pakistan may be leading to unsafe production of wheat grains. This study therefore looked into the uptake of IMI from seed treated with IMI to the growing crop of wheat, as well as the absorption of IMI in the growing crop when the insecticide was applied via foliar spray treatment.

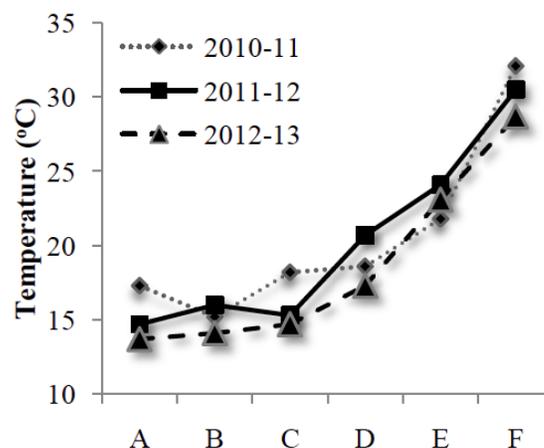


Figure 1: Variation in temperature from sowing to harvesting; (A) Sowing, (B) One month after sowing, (C) Two months after sowing, (D) Three months after sowing, (E) One month before harvesting, (F) Harvesting.

3.1. Temperature Variation during Study Period

Throughout the study period, temperatures were monitored at different stages from sowing to the harvesting. The results shown in Figure 1, indicates that at each experimental stage, average temperatures were comparable to each other from 2010-11 to 2012-13. In year 2010-11, temperatures varied between 15.2 to 32.1°C, 14.7 to 30.5°C in year 2011-12 and 13.7 to 28.3°C in year 2012-13. In all three years, the highest difference among the temperatures noted at any specific stage was the 'sowing' time when the temperatures varied (only 3.6°C) between 13.7 (in 2012-13) to 17.3°C (in 2010-11). It may therefore be concluded that the effect of temperature would have had a similar influence on the various parameters studied during the 3 years. Therefore, temperature variations during field experimental period were not considered likely to have had any significant effect on the residue levels of IMI studied in wheat grains and straw.

3.2. Residues of Imidacloprid in Grains and Straw

After harvesting, the residues of IMI in wheat grains and straw collected from all the experimental fields

were quantitatively analyzed in three replicates. The experiments were performed over three consecutive years (i.e. 2010-11, 2011-12 and 2012-13). The results of the experiments are shown in Table 1.

Table 1: Average Residues of IMI in Wheat Grains and Straw after Harvesting in Three Consecutive Years

Treatments	Commodity	Residues of Imidacloprid ($\mu\text{g g}^{-1}$)
T ₁	Grain	<LoD
	Straw	<LoD
T ₂	Grain	0.076 ± 0.002
	Straw	0.123 ± 0.025
T ₃	Grain	0.118 ± 0.003
	Straw	0.194 ± 0.030
T ₄	Grain	0.199 ± 0.006
	Straw	0.350 ± 0.016
S ₁	Grain	0.760 ± 0.017
	Straw	1.435 ± 0.094
S ₂	Grain	2.482 ± 0.102
	Straw	5.211 ± 0.434

*results are average of three replicates.
LoD = Limit of detection = 0.05 $\mu\text{g/g}$.

These results show that the grains and straw grown from seed treated with the lowest level (T₁) were found either negligible or no IMI residues. However, some residues of IMI were detected in T₂, T₃ and T₄ and were found to be in increasing order from T₂ to T₄ both in grains and straw. Particularly, at each specific treatment, these residues were found to be higher in straw than that in grains.

The results also indicate similar pattern of residues in the case of S₁ and S₂ after foliar applications. However, the lowest recommended (selected) foliar application level; S₁ = 6mg/25ft² resulted the grains and straw containing 0.76±0.071 $\mu\text{g/g}$ and 1.435±0.094 $\mu\text{g/g}$ of IMI residues respectively whereas at the lowest recommended dosage of seed treatment (T₁ = 15mg/25g of seed), both the grains and straw were found to be free (i.e. below LoD = 0.05 $\mu\text{g/g}$) of IMI residues.

3.2.1. Residues from Seed Treatments

For seed treatment T₁, the residues found both in wheat grains and straw were either not detectable or were very low. Therefore, further comparisons of IMI residues between grains and straw were made for seed treatment levels T₂, T₃ and T₄.

The results shown in Figure 2 clearly indicate that the uptake of IMI residues in straw is higher as compared to that in grains. The results also show that variation in the residue levels in grains is considerably low between the study years. The residues detected in grains among different seed treatments studied in consecutive three years were not significantly different from each other ($P>0.05$). However, in the case of straw from seed treatment T₂ and T₃ in year 2011-12 and 2012-13 were significant to each other ($P>0.05$) but not with the result obtained in year 2010-11 whereas results for T₄ in 2010-11, 2011-12 and 2012-13 were not significantly different from each other ($P>0.05$).

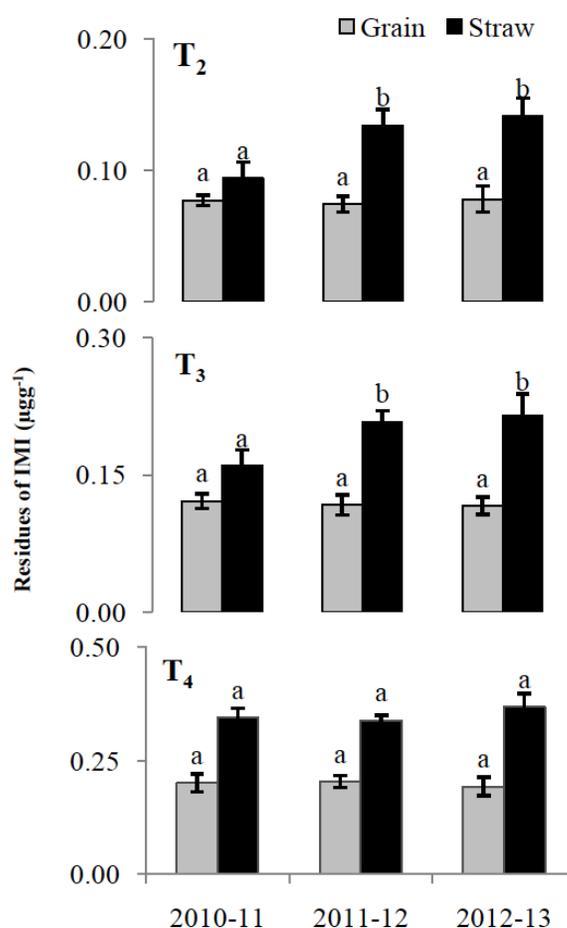


Figure 2: Residues of IMI in grains and straw at different seed treatment levels.

3.2.2. Residues from Foliar Applications

The grains and straw harvested from the crops treated with foliar applications S₁ and S₂ were also analyzed for IMI residues. Like the results obtained in the case of seed treatments, residues of IMI in straw were found to be higher as compared to that in the grains collected from S₁ and S₂ experimental fields.

Following foliar application, quantitative differences in the residues of IMI in straw and grains were also higher. More specifically, in comparison to grains, almost twice the quantity of IMI was detected in straw in all the study years and at both foliar application levels (Figure 3). The results of IMI residues in grains at all foliar application levels were not significantly different to each other ($P>0.05$). However, residues of IMI detected in 2010-11 in straw at S_1 foliar treatment was found to be slightly different (but statistically insignificant - $P<0.05$) with that in 2011-12 but not with the result obtained in 2012-13. Similarly, at S_2 , results of 2010-11 and 2012-13 were not found to be significantly different to each other ($P>0.05$), but the residues detected in 2011-12 were found to be significantly different ($P<0.05$).

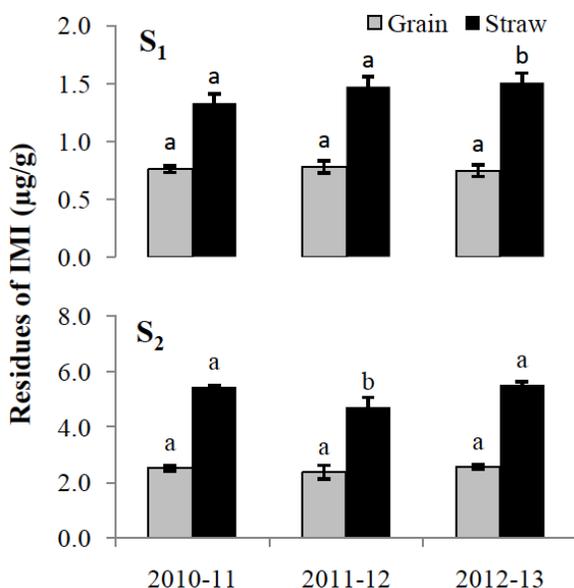


Figure 3: Residues of IMI in grains and straw at different foliar application levels.

3.3. Treatment Levels and Residual Persistence

Treatment levels were selected on the basis of the recommended application level of imidacloprid as seed treatment on wheat crop, i.e 50 to 175g /100kg seed. In this study, with the minimum recommended application level i.e. 15mg /25g of seed (T_1), no residues of imidacloprid were detected either in grains or straw. However, the crop produced by T_2 , T_3 and T_4 were found with considerable levels of IMI residues. The highest level of seed treatment (i.e T_4) was selected not on the basis of the highest recommended application level, but on the basis of the usual practice of the farmers (that is higher than the recommended level). Figure 4 shows the residual uptake of imidacloprid in wheat grains and straw with respect to

different seed treatment levels. The maximum allowable limit of IMI residues in wheat grains is $0.05\mu\text{g/g}$. In this context, all the grains produced from T_2 , T_3 and T_4 were found to exceed the MRLs. The levels of residues in straw were higher in comparison to grains at all treatment levels. Despite this, straw can be considered safe for animal feed because of a higher MRL of $1\mu\text{g/g}$ as defined by Codex Alimentarius [28].

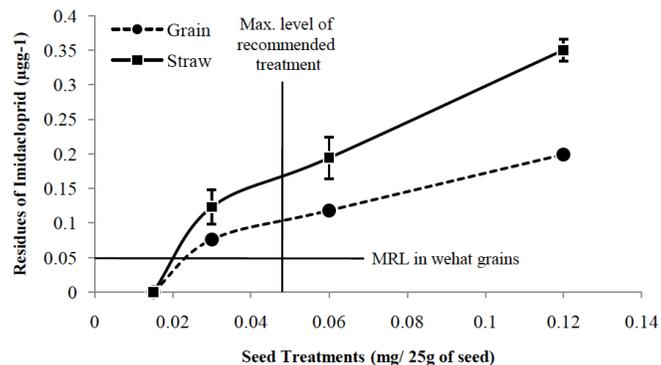


Figure 4: Residual uptake of IMI at different levels of seed treatment.

The recommended application rate of IMI as foliar application on wheat crop is 25 to 100g (active ingredient)/ha [20]. On the basis of these recommended levels, two foliar application levels i.e. $6\text{mg}/25\text{ft}^2$ (S_1) and $30\text{mg}/25\text{ft}^2$ (S_2) were selected. At both the treatments, residues of IMI in grains and straw were found to exceed the MRL (Figure 5).

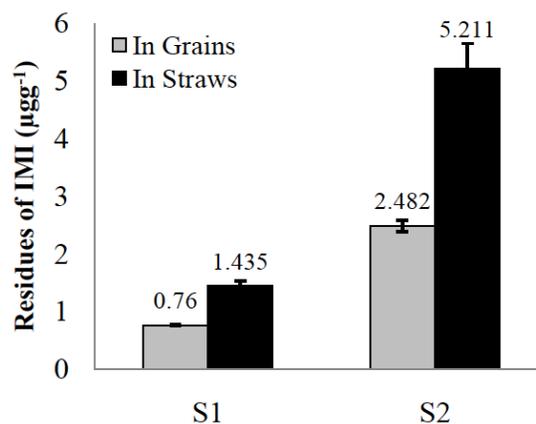


Figure 5: Residual penetration of IMI upon different levels of foliar applications.

The high accumulation of the insecticide in straw is likely to be due to large surface areas as compared to that of grains. This would allow rapid penetration and spreading of IMI residues in straw. It may also be considered that standard deviation in the case of average residues detected in straw among the three study years is somewhat higher at each seed treatment

level as compared to that in grains. This variation in residues, detected in straw among all the years may be due to the large surface area and orientation of straws in wheat plant as well as the surface area. Due to large surface area of straw, it is partially or completely under the exposure of sunlight. As the degradation of IMI is temperature dependent [29], a higher exposure of sunlight may intensify degradation of the insecticide. For this reason, the degradation pattern may also vary in different portions of the straw. On the other hand, grains are normally sheltered from the sunlight by the straw and therefore photo-degradation of IMI is likely to be relatively slower.

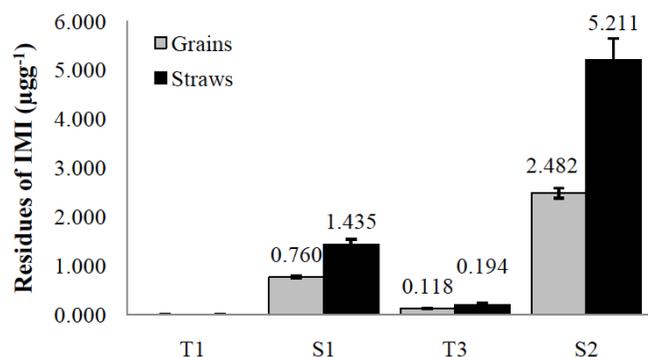


Figure 6: A comparison of recommended application levels of seed treatment and foliar application. T₁ (minimum recommended seed treatment level), S₁ (minimum recommended foliar Application level), T₃ (slightly above the maximum recommended seed treatment level), S₂ (maximum recommended foliar application level).

Upon comparing both treatment types, the detected residues in plant products were found to be higher following foliar applications. At minimum recommended levels, i.e. T₁, no residues were detected in either of the plant products analyzed. At S₁, 0.76 and 1.44 µg/g of residues were detected in wheat grains and straw respectively (Figure 6). Similarly, residues detected in plant products grown from T₃ (slightly above the maximum recommended level for seed treatment) were

very low as compared to grains and straw from S₂ field area (where maximum recommended levels of IMI were used as foliar application).

These results also enabled the estimation of the uptake of residual content in the plant's aerial parts following different treatments. The data have been shown in Table 2 which indicates that with the treatment T₃, residual uptake into the grains and straw remained 77.63% and 78.86% respectively as compared to the uptakes into the grains and straw with treatment T₂. Similarly, with reference to the treatment T₂, The residual uptakes into grains and straw grown from treatment T₄, were found to be 65.46% and 71.14% respectively. These findings clearly show that percent residual uptake into aerial parts of wheat plant decreases with the quantity of IMI used for seed treatment. This decreasing trend in residual uptake of IMI is more clearly evident in grains.

For foliar applications, percent residual absorption in grains and straw grown from S₂ field were found 65.32 and 72.63% respectively compared to the residues detected in grains and straw grown from S₁ field. These values also show that in spite of increase in percent residual degradation upon increasing the dosage levels, absorption of IMI residues in straw is higher than that in grains. Since, the straw have larger surface area compared to grains, it allows more quantity of IMI to stay on the surface of straw. This is the most likely reason why absorption of IMI was found higher in straw than in grains.

4. CONCLUSIONS

The present study was conducted to investigate the levels and persistence of imidacloprid residues in wheat grains and straw in field crops (samples) grown from treated seed and foliar sprayed crops. The aim of the study was to find out the best practices that may be

Table 2: Comparison of Residual Uptake among Different Treatments

	Treatments (in mg)	In Grains		In Straws	
		Residue (µg g ⁻¹)	% residue [*]	Residue (µg g ⁻¹)	% residue [*]
Seed Treatments	15 (T ₁)	<math><0.05</math>	<math><0.05</math>	<math><0.05</math>	<math><0.05</math>
	30 (T ₂)	0.076	N/A	0.123	N/A
	60 (T ₃)	0.118	77.63	0.194	78.86
	120 (T ₄)	0.199	65.46	0.350	71.14
Foliar Application	6 (S ₁)	0.76	N/A	1.435	N/A
	30 (S ₂)	2.482	65.32	5.211	72.63

*with reference to the T₂ and S₁ accordingly.

used for the safe production of wheat and straw with no or permissible levels of imidacloprid residues. This study was conducted in an experimental field over 3 consecutive years. The trials included crops grown from seeds treated with imidacloprid, and also foliar spray on standing crop (one month before harvest) with a commercial imidacloprid formulation (Confidor 200SL). The treatment levels were selected on the bases of minimum and maximum application levels that are recommended for seed treatment and foliar application.

The study showed that whilst residues of imidacloprid increased with an increase in application rate, straw samples were found to contain a higher level of residues compared to grains at all treatment levels. This is likely to be due to the large surface area and flat surface of straw that allows imidacloprid to penetrate more into straw than in the grains. Although field trials found grains and straw samples from treated-seed crops to contain imidacloprid residues that exceeded MRL, they were not excessively high. In comparison, quite high levels of residues were found in the grains collected from foliar application field experiments. These findings will provide scientific evidence to support decision making in regard to either restricting the use of imidacloprid to seed-treatment only, setting a safe application level for foliar application (up to four folds lower than the currently used levels), or not recommending foliar application altogether.

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