

## Potential Impacts of Climatic Changes on Indoxacarb Persistence and its Pre-harvest Interval in Tomato Fruits.

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### Abstract:

It is real and significant that climatic changes affect the pesticide behaviour, but there are no data available on their effect on the pesticide persistence and its pre-harvest interval (PHI). Therefore, to estimate the potential effect of climatic changes on the fate of indoxacarb in tomato fruits, the decline rate of indoxacarb residues was investigated in winter and summer seasons, for three consecutive years under field condition. Residues of indoxacarb were determined by high performance liquid chromatography with photodiode array detector (HPLC-DAD). Recoveries were between 89.5-96.4% with a RSD of 8-15% in tomato at spiked levels of 0.01, 0.05 and 0.1 mg kg<sup>-1</sup>, respectively. The LOQ of this method was found to be 0.01 mg kg<sup>-1</sup> while LOD being 0.005 mg kg<sup>-1</sup>. Indoxacarb was sprayed on tomato at recommended dosage. Samples of tomato were collected at 0, 1, 3, 5, 7, 10 and 15 days after treatment. The decline rates of indoxacarb were described using first-order kinetics. The results indicated that, the mean half-lives of indoxacarb in tomatoes and PHI changed significantly through all seasons of the trial. The least half-life time and PHI were 1.400 and 2.100 d in summer season of 2014, respectively, while the longest were 2.773 and 4.800 d in winter season of 2012, respectively. Also, a negative correlation coefficient was observed between the averages of air temperature (of days after indoxacarb spraying) in seasons of the experiment and the mean half-life times or PHIs. Whereas a positive correlation coefficient was obtained between averages of relative humidities and the mean half-life times or PHIs. It was concluded that both air temperature and relative humidity (as a part of climatic factors) play a great role in the interaction with indoxacarb persistence and its PHI. This work would be also helpful for the government of Egypt to establish the MRL of indoxacarb in tomato and to provide guidance on the proper and safe use of this insecticide.

**Keywords:** Indoxacarb; Pre-harvest interval (PHI); Climatic Changes; Pesticide; Tomato; Half-life time

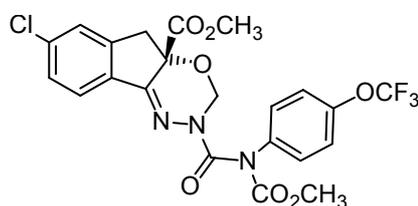
### 1. Introduction

Climate change refers to any change in climate overtime, whether due to natural variability or as a result of human activity (McCarthy et al., 2001 and Parry et al., 2007). Climate change has become a global issue in recent times manifesting in

variations of different climate parameters including cloud cover, precipitation, temperature ranges, sea levels and vapour pressure. The variations in climate parameters affect different sectors of the economy, such as agriculture, health, water resources, energy, etc. (Ogbo et al., 2013). Climate change influences the risk of pesticides leaching, since higher temperatures and increased rainfall lead to contrasting effects on pesticide leaching (Palikhe 2007; Steffens et al., 2014). The warming is important, though its intensity has varied from decade to decade, from region to region and from season to season, and been mainly caused by greenhouse gases (Crosson, 1997). Temperature has a direct and an indirect influences on the persistence of pesticides in the plants (Bedos, 2002 and Tepper, 2012). Indoxacarb, (S)-methyl 7-chloro-2, 5-dihydro-2- [[[(methoxycarbonyl) [4 -(trifluoromethoxy) phenyl] amino]carbonyl] indeno[1,2-e][1,3,4] oxadiazine-4a(3H)-carboxylate (C<sub>22</sub>H<sub>17</sub>ClF<sub>3</sub> N<sub>3</sub>O<sub>7</sub>) (Fig. 1), is a new bioactivated oxadiazine insecticide produced by DuPont in 1991. The indoxacarb racemate contains two enantiomers (S: R), designated DPX-KN128 and DPX-KN127, but only the S enantiomer has insecticidal activity (McCann et al., 2001). It has been used for controlling insect pests of cotton, fruits and vegetables in USA (Allen et al., 1999; Liu and Sparks, 1999), Australia (Holloway and Forrester, 1998), France (Olszak and Pluciennik, 1999), Italy (Bassi et al., 2000) and many other countries (Pluschkellm et al., 1998).

The tomato is one of the most popular vegetables in Egypt. Annual tomato production in the country is estimated to be seven million metric tones and area under cultivation about 221 thousand hectares which represent about 34 % of the average area of vegetables in Egypt. The tomato is considered to be an important crop and basic component of the diet and is used almost daily in Egypt. It is consumed in raw form as salad, home-cooked or processed as a sauce, juice or paste. The tomato crop is frequently infested by a number of diseases at all stages of its development (Malhat, 2013). The crop is often applied with chemical pesticides to offer protection from severe damage.

In Egypt, very limited data have been reported concerning the dissipation of indoxacarb insecticides in agricultural products and, as a result, no published data are available concerning effect of any climatic changes on the fate of indoxacarb in tomatoes. Consequently, the present investigation was carried out to estimate impacts of climatic changes on indoxacarb dissipation and its PHI in tomatoes, in three consecutive years, and to recommend and enhance safe usage of the insecticide to the growers.



**Fig. 1.** Chemical structure of indoxacarb

## 2. Materials and Methods

### 2.1. Reagent and chemicals

Indoxacarb reference standard ( $\geq 99.9\%$  purity) was purchased from Dr. Ehrestorfer Augsburg, Germany. All organic solvents used in this study were of HPLC grade

and purchased from Scharlau (Barcelona, Spain). The suitability of solvents was ensured by running reagent blank along with actual analysis. Sodium chloride of analytical grade was obtained from El Naser Pharmaceutical Chemicals Co. (Cairo, Egypt). Anhydrous magnesium sulfate of analytical grade, purchased from Merck (Germany), was activated by heating at 400°C for 4 h in muffle furnace, then cooled and kept in a desiccator before use. Primary secondary amine (PSA, 40 µm Bondesil) was obtained from Supelco (Bellefonte, PA).

## 2.2. Preparation of standard solutions

The stock solution containing 1,000 µg/ml of the analyte was prepared using acetonitrile as solvent. The standard solutions used for fortification of the matrices and instrument calibration purposes were prepared by serial dilution. All standard solutions were stored at 4°C before use. The standard calibration curve of indoxacarb was constructed by plotting analyte concentrations versus peak area.

## 2.3. Field experiment design

The experiment was conducted in Etai El-Baroad Agricultural Research Station, El-Beheira, Egypt, in winter and summer seasons, for three consecutive years. Tomato seedling [*Lycopersicon esculentum* Mill.) cv. Malika] was planted in each winter season, on 2<sup>nd</sup> October in 2011, 2012 and 2013, and on 15<sup>th</sup> March in 2012, 2013 and 2014 in each summer seasons. The experiments were designed in the following ways: plot size, 7 x 6 m; plot to plot distance, 1.5 m; plant to plant distance, 0.4 m for row to row distance 1 m. Treatment plots were arranged in a randomized complete block design with three replications. Irrigation and fertilization were made according to the crop schedule. The plots had not been treated with indoxacarb in the past. To ensure the reliability of the experimental results, the field trials were previously investigated to be free of the pesticide. Treatments were carried out using a knapsack sprayer motor. Avant®15% SC was the commercial formulation applied at the dose recommended by the manufacturer (4 g a.i./100L). The spray volume taken was 200 L/ feddan (one feddan = 0.42 ha). Untreated control tomatoes were sprayed with water. Indoxacarb was sprayed on 4<sup>th</sup> December, in each year in winter seasons, and on 30<sup>th</sup> June in each summer seasons of the experiment. Tomato samples were collected 0 (1 hour after treatment), 1, 3, 5, 7, 10 and 15 days after each treatment. Immediately after picking, the samples were put into polyethylene bags and transported to the laboratory in an ice box, where they were chopped and thoroughly mixed. The sample was kept deep-frozen (-20 °C) until analysis. Control samples were obtained from the control plots. During the trials, the average minimum/maximum daily air temperatures and the average relative humidity (of days after indoxacarb spraying) were obtained from Meteorological Station of Etai El-Baroad. There was no rainfall at any time during the experimental period.

## 2.4. Analytical methods

### 2.4.1. Sample extraction

The samples were comminuted using the laboratory blender and representative homogenized (15 g) of each was then placed into 50 ml polyethylene tube. Samples were extracted and cleaned up immediately after sampling. 15 ml of acetonitrile was added into each tube. The samples were well shaken using a vortex mixer at

maximum speed. Afterwards, 6 g of anhydrous magnesium sulphate and 1.5 g of sodium chloride were added, then extract by shaking vigorously on vortex for 5 min and centrifuged for 10 min at 4,000 rpm. An aliquot of 4 ml was transferred from the supernatant to a new clean 15-ml centrifuge tube containing 100 mg PSA and 600 mg anhydrous magnesium sulphate. The samples were again vortexed for 3 min and then centrifuged for 10 min at 4,000 rpm. An aliquot of 2 ml was filtered through a 0.2 µm PTFE filter (Millipore, USA). The sample was then ready for the final analysis in the LC system.

#### 2.4.3. Liquid chromatographic analysis

HPLC analysis was performed with an Agilent 1100 HPLC system (USA), with quaternary pump, manual injector (Rheodyne), thermostat compartment for the column and photodiode array detector. The chromatographic column was C<sub>18</sub> Zorbax XDE (250 mm x 4.6 mm, 5 µm). The column was kept at room temperature. The flow rate of the mobile phase (acetonitrile / water = 50/50 v/v) was 0.8 ml/min., and injection volume was 20 µl. The detection wavelength for detection of indoxacarb was set at 225 nm. The retention time of indoxacarb was about 3.49 min. Residues were estimated by comparison of peak areas of standards with that of the unknown or spiked sample run under identical conditions

#### 2.4.3. Validation study

The method was subjected to the validation study before its application to determining the insecticide indoxacarb residues in the samples. Recovery assays were carried out on samples of untreated tomato which spiked with the target compound at three concentration levels in five replicates (Table 1). The method trueness and precision parameters in terms of average recovery and relative standard deviation were calculated and assessed according to the European Union guidelines (SANCO/12495/2011). The linearity of the chromatographic response was evaluated over the range between 0.01 and 2 mg kg<sup>-1</sup> at five concentration levels.

#### 2.5. Calculation of half-life periods

Half-life time ( $t_{1/2}$ ) of indoxacarb was calculated mathematically according to Moyer *et al.* (1987). The dissipation kinetics of indoxacarb residues were determined by plotting residue concentration against elapsed time after application, and equation of best curve fit with maximum coefficients of determination ( $R^2$ ) was determined. For dissipation of targeted insecticide in tomato, exponential relationship was found to be applicable corresponding to the general first-order kinetics equation:

$$C_t = C_0 e^{-kt}$$

Where  $C_t$  represents the concentration of the pesticide residue at the time of  $t$ ,  $C_0$  represents the initial deposits after application and  $k$  is the constant rate of pesticide disappearance per day. From this equation, the dissipation half-life periods ( $t_{1/2} = \ln(2)/k$ ) of the studied insecticide.

#### Statistical analysis

Data were subjected to analysis of variance (ANOVA) followed by least significant difference (CoStat Statistical Software, 1990).

## Results

### 3.1. Linearity, recovery and detection limits

To ensure quality of the insecticide residue results, the method performance characteristics were generated and evaluated before real tomato samples were analysed. The average recovery and relative standard deviation (RSD) data of indoxacarb from spiked samples are detailed in Table 1. As seen, the recoveries were in the range between 89.5% and 96.4% with associated RSDs not exceeding 15%. These results are considered to be highly satisfactory for the purpose of pesticide residue analysis and they are compliant with the European Union criteria which stipulate the average recoveries in the range 70-120% with corresponding RSD less or equal 20% (SANCO/12495/2011). The limit of quantification (LOQ) of the method was defined as the lowest spiking level for which the validation criteria were satisfied and it was equal 0.01 mg kg<sup>-1</sup>. Excellent linearity with the coefficient of determination ( $R^2$ ) > 0.99 was achieved for the studied insecticide when using standard in the extract of tomato matrix (matrix-matched standard).

**Table 1** Recoveries and relative standard deviation (RSD) of indoxacarb in tomato at various fortification levels

Fortified level (mg kg <sup>-1</sup> ) (n*=5)	Recovery (%)	RSD (%)
0.01	89.5	15
0.05	96.4	8
0.1	92.6	13

\* number of replicates

### 3.2. Decline of indoxacarb residues in tomato fruits

In general, the results showed that the residue of indoxacarb in tomato fruits was varied significantly not only between winter and summer seasons but also in some cases between winter and winter seasons or between summer and summer seasons (when compared the corresponding days after insecticide spraying). In winter seasons, data indicated that indoxacarb residues were the lowest in year 2013, while they were highest in 2012, followed by 2011. The residues ranged from 1.917 to 0.020 mg kg<sup>-1</sup>. It is clear that, they varied significantly after seven days of spraying through all years of the experiment. In summer seasons, indoxacarb residues were the lowest in year 2014, while they were highest in 2012, followed by 2013. The residues ranged from 1.749 to 0.010 mg kg<sup>-1</sup>. The results also revealed that the indoxacarb residues were significantly lower in summer seasons than in winter seasons. The calculated half life times of indoxacarb were significantly changed through all years of the study whether in winter or summer seasons. The half life periods ranged from 2.773 d in winter 2012 to 1.400 d in summer 2014. As well as the pre-harvest intervals (PHIs) were significantly different during all seasons of the trial. They were longer in winter seasons (ranged from 4.8 to 4.0 d) than in summer seasons (ranged from 2.6 to 2.1 d) (Table 2).

### 3.3 Climatic change of temperature and humidity

The averages of air temperatures and the relative humidities were fluctuated, during the days after spraying of indoxacarb in all tested seasons. In winter seasons, the mean

air temperatures ranged from 19.8 (in 2012) to 21.0 °C (in 2013), while the relative humidity ranged from 82.2 (in 2011) to 63.6 % (in 2012). Whereas, in summer seasons, the main air temperatures ranged from 34.3 °C (in 2012) to 38.3 °C (in 2014), while the relative humidity ranged from 79.1 (in 2012) to 64.7 % (in 2014) (Table 3).

#### 3.4 Correlation coefficient between the air temperature or relative humidity and indoxacarb half lives or pre-harvest intervals (PHI) of tomato fruits

The correlation coefficient was a negative between the averages of air temperatures (of the days after indoxacarb spraying of each season in the experiment) and both the half life times of indoxacarb and PHIs, with values of -0.972 and -0.994, respectively. However, it was a positive between the averages of relative humidities and both the half life times of indoxacarb and PHIs, with values of 0.212 and 0.241, respectively (Table 4).

Table 2 Residues of indoxacarb in tomato fruits

Time after application (Day)	Residues of indoxacarb in tomato fruits (mg kg <sup>-1</sup> )						L.S.D
	Seasons						
	Winter			Summer			
	Year						
	2011	2012	2013	2012	2013	2014	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Z	1.853 <sup>a</sup> ±0.23	1.917 <sup>a</sup> ±0.28	1.831 <sup>ab</sup> ±0.24	1.749 <sup>bc</sup> ±0.31	1.697 <sup>c</sup> ±0.19	1.663 <sup>c</sup> ±0.25	0.101
1	1.151 <sup>ac</sup> ±0.21	1.221 <sup>ab</sup> ±0.35	1.113 <sup>bc</sup> ±0.19	0.990 <sup>d</sup> ±0.20	0.890 <sup>e</sup> ±0.15	0.844 <sup>e</sup> ±0.16	0.088
3	0.782 <sup>a</sup> ±0.19	0.813 <sup>a</sup> ±0.23	0.760 <sup>a</sup> ±0.05	0.420 <sup>b</sup> ±0.12	0.364 <sup>bc</sup> ±0.10	0.312 <sup>c</sup> ±0.08	0.057
5	0.434 <sup>ab</sup> ±0.13	0.470 <sup>a</sup> ±0.12	0.402 <sup>b</sup> ±0.15	0.180 <sup>c</sup> ±0.10	0.104 <sup>d</sup> ±0.04	0.091 <sup>d</sup> ±0.03	0.042
7	0.238 <sup>a</sup> ±0.07	0.292 <sup>b</sup> ±0.09	0.201 <sup>c</sup> ±0.05	0.080 <sup>d</sup> ±0.03	0.071 <sup>d</sup> ±0.03	0.060 <sup>d</sup> ±0.03	0.022
10	0.083 <sup>a</sup> ±0.02	0.092 <sup>b</sup> ±0.02	0.072 <sup>c</sup> ±0.01	0.030 <sup>d</sup> ±0.01	0.019 <sup>e</sup> ±0.01	0.010 <sup>f</sup> ±0.004	0.004
15	0.040 <sup>a</sup> ±0.03	0.050 <sup>a</sup> ±0.04	0.020 <sup>b</sup> ±0.01	ND	ND	ND	0.018
HL (Day)	2.646 <sup>a</sup> ±0.21	2.773 <sup>b</sup> ±0.35	2.295 <sup>c</sup> ±0.14	1.707 <sup>d</sup> ±0.19	1.565 <sup>e</sup> ±0.13	1.400 <sup>f</sup> ±0.15	0.083
PHI (Day)	4.5 <sup>a</sup> ±0.28	4.8 <sup>b</sup> ±0.36	4.4 <sup>c</sup> ±0.17	2.6 <sup>d</sup> ±0.14	2.3 <sup>e</sup> ±0.19	2.1 <sup>f</sup> ±0.22	0.095

Z: two hours after the insecticide application (zero time).

HL: Half-life time (t<sub>1/2</sub>)

PHI: Pre-harvest interval

ND: Not detected.

Values within the same row having the same letters are non-significant, p<0.05.

SD: Standard deviation

**Table 3** The mean air temperatures (°C) and relative humidities (%) for days after spraying of indoxacarb

Year	Seasons			
	Winter		Summer	
	Temperature mean±SD	Humidity mean±SD	Temperature mean±SD	Humidity mean±SD
2011	20.4±0.8	82.2±4.4	-	-
2012	19.8±1.0	63.6±5.5	34.3±1.1	79.1±3.0
2013	21.0±1.0	76.9±9.0	34.7±1.2	64.7±3.9
2014	-	-	38.3±3.0	67.9±7.4

SD: Standard deviation

**Table 4** Correlation coefficient between averages of air temperatures or relative humidities (for the days after spraying) and indoxacarb half lives or pre-harvest intervals of tomato fruits (PHI) during six seasons of the experiment.

Climatic parameter	Correlation coefficient	
	HL	PHI
Temperature	-0.972	-0.994
Humidity	0.212	0.241

HL: Half-life time ( $t_{1/2}$ )

PHI: Pre-harvest interval

### Discussion

Significant research effort has been dedicated to understanding the fate of pesticides in the environment, and the relationships between pesticide fate and specific environmental parameters are generally understood at least qualitatively. The influence of the climatic changes on pesticide persistence is extremely complex, because not only do they affect most of the chemical and plant factors, but they also interact with each other. Current best estimates of changes in climate indicate an increase in global mean annual temperatures of 1°C by 2025 and 3 °C by the end of the next century (Palikhe 2007). In the present work, it was examined the potential impacts of climate change on indoxacarb persistence and pre-harvest intervals of tomato fruits (PHIs). The results clearly indicated that the residues of indoxacarb in tomato fruits (when compared the corresponding days after insecticide spraying) changed significantly from winter season to the summer season and through the same season in some years of the trial. Despite the residues of indoxacarb were the higher in winter and summer seasons of 2012, the lowest residues were in 2013 for winter

seasons and in 2014 for the summer seasons. Consequently, the half-life time of indoxacarb in tomato fruits decreased significantly to the shortest in summer season of 2014, and it was the longest in winter season of 2012. Also the pre-harvest intervals of tomato fruits (PHIs) reduced significantly to the shortest period in summer season of 2014, and it was the longest in the winter of 2012. On the other hand, the average recorded of the air temperature through the days after indoxacarb spraying was the highest in summer season of 2014, and the lowest in winter season of 2012. Furthermore the correlation coefficient was a negative between the averages of air temperatures of the days after indoxacarb spraying and the half life times of indoxacarb or PHIs. Whereas, it was a positive between the averages of the relative humidities and the half life times of indoxacarb or PHIs. In a previous study, Tepper (2012) found that the temperature has a direct influence on the uptake of pesticides and on the rate at which pesticides volatilise from plant surfaces. It also has an indirect influence on the evaporation of the aqueous component of pesticide droplets. Also, higher temperature increases volatilisation rates and promotes the transfer of vapours into the atmosphere from plants. Volatilisation may represent a major dissipation pathway for pesticides applied to crops. It accounts for up to 90% of the applied dose in some cases (Bedos, 2002). Also, the rate at which spray droplets evaporate is determined by the amount of water vapour that the air can absorb. The absorption potential is dictated by the amount of water vapour already in the air and the temperature of the air. Relative humidity is the ratio of the actual amount of water vapour in the air to the amount it could hold when saturated. The air's capacity to hold water vapour increases as air temperature increases. At 30°C, the capacity is more than three times that at 10°C; consequently, while the amount of water vapour in the air may be static, the relative humidity decreases as temperature increases. A better indicator of the rate at which pesticide droplets evaporate is Delta T. Delta T is the difference between the wet and dry bulb temperatures. It combines the effects of temperature and relative humidity (Tepper, 2012). Otherwise, the residue pattern of indoxacarb in tomatoes under different treatments have followed a trend in which shorter harvest intervals led to higher residue level. FAO/WHO had established MRLs of 0.5 mg kg<sup>-1</sup> for indoxacarb in tomatoes (Codex, 2013). In the present study, the residues of indoxacarb were above the prescribed MRL (0.5 mg kg<sup>-1</sup>), on day 2 in the summer season or on day 4 during winter season and below the MRL on day 3 (in summer season) or on day 5 (in winter season). These results suggested that it is safe to harvest 3 days in summer season or 5 days in the winter season when applying the recommended dose of indoxacarb. In light of the present findings, weather variability and climate change affect the persistence of the pesticide and its PHI. These issues should be seriously considered when to recommend and promote safe usage of pesticide to the growers.

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## المخلص العربى

تمت هذه الدراسة لدراسة تأثير التغيرات المناخية على ثبات متبقى مبيد الاندوكساكارب ومعرفة فترة ما قبل الحصاد خلال الموسمين HPLC (DAD) الشتاء والصيف فى الفتره من 2011 الى 2014 على محصول الطماطم صنف مالিকা وتم تقدير المتبقى بجهاز وكانت نسبة الاسترجاع 96,4-89% وذلك خلال الفترات من صفر و 1 و 3 و 5 و 7 و 10 و 15 يوماً بعد المعاملة . وكانت أعلى درجة حرارة في موسم 2014 خلال فصل الصيف وكانت فترة نصف العمر للمبيد 1,4 يوم وفترة ما قبل الحصاد 2,1 يوم بينما كانت أقل درجة حرارة في موسم 2012 خلال فصل الشتاء وقد سجلت فترة نصف العمر للمبيد 2,773 يوم وفترة ما قبل الحصاد 4,8 يوم وقد تمت مقارنة نتائج المتبقى بالـ MRL (الكودكس) الخاصة بالمبيد وهي 0,5 مللجرام / كجم.