RISK ASSESSMENT OF CERTAIN PESTICIDES USED ON GRAPES UNDER THE EGYPTIAN ENVIRONMENTAL CONDITIONS

By

SHAIMAA MOHAMMED SAYED

B.Sc. Agric. Sc. (Pesticides), Ain Shams University, 2012

A Thesis Submitted in Partial Fulfillment

Of

The Requirements for the Degree of

MASTER DEGREE

in

Agricultural Sciences

(Pesticides)

Department of Plant Protection

Faculty of Agriculture

Ain Shams University

2019

Approval Sheet

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ABSTRACT

Shaimaa Mohamed Sayed Mohamed: Risk Assessment of Certain Pesticides Used on Gapes under the Egyptian Environmental Conditions. Unpublished MS.c Thesis, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, 2019

1. **Residues of Imidacloprid and Myclobutanil in/on Grape and Soil under Field Conditions:**

Persistence Vs degradation behavior of insecticide imidacloprid (35% SC) and fungicide myclobutanil (24% EC) in/on grape (leaves and fruits) and surrounding soil under the canopy were investigated under field conditions. Leaves, fruits and soil samples were collected at 2 hours to 21 days after application at the recommended rate. QuEChERS method was used for extraction and clean-up and analyzed using HPLC and GC for imidacloprid and myclobutanil, respectively .The initial residue deposits, degradation percentages and/or, the parameters (RL_{50} and RL_{90}) and Pre Harvest Intervals (PHIs) of the targeted pesticides were the criteria of concern. Results revealed that grape leaves retained higher initial amounts than fruits by about 5.0 7 and 1.34 times for imidacloprid and myclobutanil, respectively. As for RL_{50}, RL_{90} and PHIs values, imidacloprid showed 4.12 ،13.42 and 21.95 days and 5.13 ،13.41 and 11.96 days on grape leaves and fruit, respectively. The corresponding calculated values were 4.71, 9.38 and 16.31days and 1.97, 9.14 and 14.90 days for myclobutanil on the same targeted samples, respectively .In addition, the grapefruits could be consumed safely after 12 and 15 days of treatment with imidacloprid and myclobutanil, respectively. On the other hand ،results indicated that the residue half-life **(**RL_{50}) values for the same targeted pesticides in soil were 11.56 and 15.74 days, respectively. In general, myclobutanil residues in soil recorded higher persistence levels than higher imidacloprid and on the contrary, it showed less persistence in/on grape leaves and fruits.

2. **Cytotoxicity of Imidacloprid and Myclobutanil Pesticides on Three Cancer Cell Lines:**

Three cancer cell lines, i.e. HEpG-2 (human liver carcinoma), MCF-7 (human breast adenocarcinoma), and PC3 (Prostatic Small Cell Carcinoma) were used to determine the cytotoxic effects of the neonicotinoid insecticide (imidacloprid) and conazole fungicide (myclobutanil). Cytotoxicity was measured by neutral red incorporation (NRI) assay. The lowest concentration of the tested pesticides (0.5 µg/ml) was toxic. With the increase of the concentration up to 80 µg/ml, the damage degree of the cellular form and size was more serious. The midpoint cytotoxicity value, (NRI_{50}) for imidacloprid and myclobutanil for HEpG-2, MCF-7, and PC3 cancer cell lines were 110.5, 67.7 and 67.6 µg/ml and 38.12, 41 and 27.5 µg/ml, respectively. In general, myclobutanil was very toxic in the three cancer cell lines compared with imidacloprid.

3. **Genotoxic Effects of Imidacloprid and Myclobutanil on *Drosophila melanogaster*:**

The genotoxic effects and carcinogenic activity of the neonicotinoid insecticide (imidacloprid) and conazole fungicide (myclobutanil) were estimated on/in *D. melanogaster*. In this respect, the acute effects of the two tested pesticides on *D. melanogaster* adults and their genotoxic effects using the Drosophila wts-SMART test based on tumor suppressor gene, warts (wts) were measured. The values of LC_{50} for imidacloprid and myclobutanil on *D.melanogaster* adults were 13 and 37 ppm, respectively. Imidacloprid and myclobutanil treatments produced a highly significant increase in the frequency of inducing wts clone spots in both males and females (0.183, 0.154/Imidacloprid and 0.10 and 0.14/myclobutanil, respectively). In addition, the two tested pesticides have evidence of genotoxic potential using SMART assays and showed a and/or positive result. In general, imidacloprid shows highly genotoxic effects compared with myclobutanil, whereas both pesticides showed high carcinogenic activity and the probability of potential risk to humans.

ACKNOWLEDGMENTS

Firstly, my full gratitude is to the one and the only almighty Allah who helped me to complete my Master degree.

All thanks and praise to my supervisor Prof. Dr. Mohamed Ibraheam Abdel-Megeed, Professor of Pesticides Chemistry and Toxicology, Faculty of Agriculture, Ain Shams University. Who without his devotion, dedication and guidance, this thesis would have not been achieved. Thank you for being so kind and supportive.

I would like to thank Prof. Dr. Khaled Mohamed Allam, Professor of Pesticides Chemistry and Toxicology, Faculty of Agriculture, Ain Shams University. Who have been always approachable, helpful and without his guidance, this thesis would have not been achieved.

My gratitude to Dr. Maher Abdel-Alem Hammad, lecturer of Pesticides Chemistry and Toxicology, Faculty of Agriculture, Ain Shams University. For his substantive guidance that influenced this thesis.

Dr. Naglaa Mohamed Ebeed, Professor of Genetics, Faculty of Agriculture, Ain Shams University. For her contribution, guidance and assistance in genetics practical work by providing laboratory, equipment, Drosophila flies under her supervision and guidance and writing researchs and finalizing this thesis.

My fully thanks and gratitude to Prof. Dr. Alaa Eldin Bayoumi, for his Contribution, encouragement, assistance, knowledge and his judging for his research with a high degree of accuracy and attention.

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INTRODUCTION

The use of pesticides is one of the most effective tactics used for controlling agricultural pests. Pesticides significantly contribute to increasing of crop productivity. Environmental contamination by pesticides can occur by means of volatilization, leaching, adsorption, absorption in soil and runoff (**Sigrh 2005**).

Since the mid of the 1990s, the class of neonicotinoids has become the most widely used and fastest growing family of insecticides worldwide (**Buckingham *et al*. 1997; Tomizawa and Casida 2005; Brown *et al*. 2006; Jeschke *et al*. 2011**). Development of new pesticides, such as neonicotinoid family includes imidacloprid, are used against severe pests with the lowest negative environmental impact (**Ambrose 2003**).

Imidacloprid (IMI), [1-(6-chloronicotinyl)-2-nitroimino-imidazolidine] is selective pesticide against the target species and show less toxic effects to non-target organisms (NTO) compared to other insecticide groups (**Casida and Quistad 2004**; **Jeschke *et al*. 2011**). The lower toxicity of IMI may be due to its interaction with the nicotinic acetylcholine receptors (nAChRs) of the central nervous system. They target and bind to postsynaptic nAChRs of insects, hence they induce a neuronal hyper-excitation and accumulation of acetylcholine, leading to the insect's death within minutes (**Buckingham *et al*. 1997; Matsuda *et al*. 2005; Tomizawa and Casida 2005**).

Myclobutanil is a systemic conazole class fungicide widely used as an agrochemical, which was introduced in the 1980s, for controlling several diseases such as powdery mildew, *Erysiphe necator*; (*Uncinula necator*) Downey mildew, *Plasmopara viticola* on fruit, vegetables, and seed commodities in the EU and elsewhere to control fungi such as Ascomycetes, Fungi Imperfect and Basidiomycetes. Its widespread use has raised the issue of possible health risks for agrarian communities and

the general population, which can be exposed to residues present in food and drinking water. The toxicities identified include adverse effects on liver and kidney and on the development of male reproductive organs. Also it has suspected endocrine disrupting properties (**Antonietta *et al*. 2016; Maarke *et al*. 2014; Fishel. 2005).**

In general scope, the intensive and increase the use of pesticides as a response to the continued efforts to intensify crop production has consequently become an invertible and controversial issue resulting in serious problems as regards to the target pests and adverse effects against environmental non-target organisms and human health. Nowadays, the cultivation of grapes is widely spread around the world with an estimated surface area of 7.6 million hectares in 2014 **(Grimalt and Dehouck 2016).** Grapes a nutritionally important fruit crop of international trade significance is consumed both as fresh and processed products **(Sinha *et al.* 2012).**

Imidacloprid is a relatively new insecticide with high activity against sucking insects. It is the most use systemic insecticide in the world in more than 100 countries **(Bonmatin *et al.* 2003)** especially on grapes, Myclobutanil belongs to conazole fungicide and it is a systemic fungicide with preventive, curative and eradicates properties for grape fungi. On the other hand environmental fate is the backbone of hazard evaluation components in the risk assessment system. However, the wide spectrum of contamination with pesticide residues as one of the main elements of our ecosystem and environment was reported by many investigators **(Shady *et al* 2000).**

A wide range of factors determines the fate of pesticides in the environment. These include chemical characteristics (vapor pressure, solubility, and adsorptive behavior), environmental characteristics (precipitation, temperature and soil, sediment and water characteristics) and agricultural practices (cropping practices, application methods, the timing of application and landscape). Risk assessment regulatory

decisions relating to the approval of pesticides are based on whether the predicted levels of exposure following the proposed or approved use are safe to consumers, operators and the environment. Although environmental exposure depends largely on the behavior of the pesticide in the field, this cannot be defined simply by factors such as degradation rate and mobility. It has been reported that soil pH**,** moisture, temperature and pesticide concentration are the most important factors affecting the persistence of pesticides **(Baskaran *et al.* 1999)**. These factors are highly dependent on environmental conditions such as temperature, rainfall and soil properties and therefore can only be quantified for specific laboratory or field studies. Degradation can include such processes as hydrolysis, photolysis, microbial metabolism, etc. Movement reduces the concentration in the treated compartment but transports residues to untreated compartments, e.g. from plant surface to soil or soil to water. A stepwise or tiered approach allows an efficient selection of tests essential to each individual risk assessment **(Shady *et al.* 2000 and Sinha *et al.* 2012).**

The use of cell lines along with bio-monitoring data could enable a proper understanding of environmental metal/chemical holistically **(Nakadai *et al.* 2006)**. Several toxicology test animal systems are undependable, as the exemplary mouse or rabbit or guinea pig systems are not true representatives of the human body. Therefore, there are large gaps in the mirror of toxicity models with animals and humans **(Tzimas *et al.* 1997)**. Nonetheless, all popular animal models used in toxicology are mammals. Further, the requirement of a high number of animals in toxicity studies is so much that, for a myriad of test chemicals like 30,000 or more in number, discourages the use of whole animals in assay systems **(Gilbert *et al.* 2010).** Therefore, the use of cell lines in toxicology had been well recognized **(Rosler *et al.* 2004)**. However, whole animal tests can never be adequately standardized like human cellular systems, as the experiment should have growing cells under controlled conditions, for example, in vitro-cultured human cell lines.

INTRODUCTION

Some chemical structures of pesticides were proved to exert mutagenic effects through various genotoxic mechanisms in deferent organisms including human beings (**Suhasini and Brosh 2013; Prashanth *et al.* 2015**). Mutagenic chemicals are also able to induce cancer and this problem has driven a maximum of the mutagenicity testing applications. The identification of such materials capable of inducing mutations has emerged as a crucial system in safety assessment. The assessment estimates the potential adverse health consequences of exposure to toxic. The issue of risk assessment of genotoxic and carcinogenic substances is relevant for chemicals used or present in foods, non-foods and in several industrial applications. Earlier studies have reported that some pesticides have mutagenic and clastogenic effects in several biological test systems (**Stivaktakis *et al*. 2010; Malik *et al*. 2012; Moulas *et al.* 2013**).

Neonicotinoids are one of the recent, effective and widely used class of neuroactive insecticides worldwide, a novel mode of action and reduced environmental hazards. (**Maienfisch *et al.* 2001; Tomizawa and Casida 2005**). The persistence of neonicotinoids increases the duration over which non-target organisms may be exposed (**Krupke *et al*. 2012; van der Sluijs *et al*. 2013**). Due to the presence of a negatively charged nitro or a cyano group, neonicotinoids have a much lower affinity for mammalian nAChRs and corresponding low toxicity to humans (**Tomizawa and Casida 2005**). Low levels of neonicotinoids cause negative effects on aquatic ecosystems both at the individual and population level (**Pisa *et al*. 2015**).

However, imidacloprid was found to induce DNA damage in a dose-related manner in earthworms as well as to increase the frequency of adducts in calf thymus DNA indicating agent-induced genotoxicity (**Shah *et al.* 1997; Zang *et al*. 2000**). Also, IMI promoted hypoglycemia, oxidative stress damage and DNA damage in different tissues of the Neotropical fish *Prochilodus lineatus* (**Vieira *et al.* 2017**). Imidacloprid