DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

Larbi Tébessi university-Tebessa

Faculty of Exact Sciences and Natural and Life Sciences

Département : *Applied Biology*

MASTER'S THESIS

Field : *Natural and Life Sciences*

Faculty : *Biological science*

Option : *toxicology*

THEME :

Protective role of Melissa sp extract on neurological impacts of imidiacloprid in Wistar rats.

Presented by:

Salemi Mohcen & Brahimi Rihem

Gury members:

Boussekine Samira	**Dr.**	**University of Tébessa**	**President**
Rachid Rouabhi	**pr.**	**University of Tébessa**	**Supervisor**
Menaceur F	**Dr**	**University of Tébessa**	**Examiner**

Soutenances date: 15/06 /2020

ملخص:

النيونيكوتينويد هي ماده كيميائية موجودة في العديد من المبيدات الحشرية، يتم استخدامها بشكل رئيسي في الزراعة والتي نميز من تركيبتها " ايميداكلوبريد" ، يلعب هدا الأخير دورًا في التأثير على الجهاز العصبي المركزي بالتحديد في الدماغ ، من ناحية أخرى ، نبات الحبق هو نبات يستعمل في العلاج الطبيعي متواجد بكثره في حوض البحر الأبيض المتوسط.

الهدف من دراستنا هو معرفة تأثير الايميداكلوبريد على المكونات البيوكيميائيه للدماغ مثل الكربوهيدرات ، البروتينات الكليه الدهون، وعلى مستوى GSH و MDA أيضًا على الأنشطة الإنزيمية GST, GPX, CAT و AChE ،حيث قمنا باستعمال جرعتين مختلفتين (5ملغم / كغم / يوم و 50 ملغم / كغم / يوم) واهتمت دراستنا بخصوص زياده حجم و نفاذيه الميتوكوندري والتنفس الخلوي.

في الوقت نفسه، قمنا بدراسة جرعات مختلطة من النيونيكوتينويد بالاضافه الى جرعه مستخلص ميليسا لنتحقق من القيمة العلاجية لهذه النبتة، ويمكننا بذلك ان نعتبرها ترياقًا للأمراض العصبية.

النتائج التي تم الحصول عليها ان الايميداكلوبريد له الدور في زيادة نسبة معلمات الإجهاد التأكسدي .MDA :البروتين ؛ GST و الانشطه علي مستوى الميتوكوندري و الانخفاض في نسبه الدهون الكليه و ,AChE, GPx GSH بخصوص الاختبارات السلوكية سجلنا فروقات معتبرة نسبيا

الكلمات المفتاحية: النيونيكوتينويد, المبيدات الحشرية, الايميداكلوبريد, الجهاز العصبي ,الأنشطة الانزيمية, نبات الحبق

Abstract:

Neonicotinoids are chemicals found in many insecticides, we use them especially in agriculture whose which to distinguish the substance imidacloprid, , the latter plays an affective role on the central nervous system specifically the brain, on the other hand the Melissa officinalis is a medicinal plant very known in the Mediterranean basin. The objective of our study is to make known the effect of imidacloprid by 2 varied doses (5 mg / kg / day and 50 mg / kg / day), on the brain metabolites such as carbohydrates, total protein lipids, on the rate GSH and MDA also that on the enzymatic parameters GST GPX CAT and AChE, we were interested in the swelling, the mitochondrial periiablity, and the cellular respiration, At the same time, we are studying mixed dosages with Melissa to reach the therapeutic curative value so we consider it as an antidote for neurological diseases.

The results obtained showed that imidacloprid has a role in increasing the ratio of oxidative stress parameters: MDA. Protein; GST and activities at the mitochondrial level and the decrease in the percentage of total fat and GSH GPx, AChE, regarding behavioral tests, we recorded relatively significant differences.

Key words: neonicotinoids, pesticides, imidacloprid, nervous system, enzymatic activities, melissa plant.

Resume:

les neonicotinoides sont des produits chimiques contenus dans nombreux insecticides, on les utilise surtout dans l'agriculture dont les quelle on distingue la substance imidaclopride , cette dernière joue un rôle d'affectation sur le system nerveux centrale precisement le cerveau ,en revanche la Melissa officinalis est une plante medicinale tres connue dans le bassin mediterraneen.

L'objectif de notre etude est de faire connaitre l'effandd'imidaclopride par 2 dose variee (5mg /kg/j and50mg/kg/j) , sur les metabolites encephalique tel que les glucides, lipides proteines totaux , sur le taux GSH andMDA aussi que sur les paramètres enzymatique GST GPX CAT andAChE ,on etait interesse par le gonflement ,la permiablite mitochondriale ,andla respiration cellulaire .parallelemnt ,on fait l'etude des dosage mixte au Melissa pour atteindre la valeur curatif therapeutique donc on la considere comme antidote pour les maladies neurologique.

Les resultats obtenus signifie que le gavage des rats par limidaclopride est cause augmentation aux niveau des parametres de stress oxydatif andmetabolites : MDA.protein ;permiabilte andgonflement mitochondriale par ailleur une dimunision au niveau lipidique GPX ,GSH,AChE aussi que les differences apparut dans les tests comportementaux d'autre part les doses mixtes fait une neutralisation au niveau des parametres precedentes

Mots cles: neonicotinoïdes, pesticides, imidaclopride, système nerveux, activites enzymatiques, plante melissa.

Acknowledgment

My big and first thanks go to ALLAH for his blessing and his kindness to me, I am grateful to ALLAH for facilitating my way to reach all my dreams.

I would like to express my deep gratitude to my supervisor Dr. Rouabhi Rachid. I would like to thank him very much for his constant confidence in me and for his faith in my abilities. I am grateful to him for all the experience I have gained during the preparation of this work. I really thank him for his great patience and continued encouragement during this study. It was a dream to beunder his influence so I thank him for achieving my dream.

I would like to thank my thesis committee: Dr Bousekine Samira, Dr Menaseur Fouad for honouring them as members of the discussion committee and enriching them for my work with their constructive questions and comments. I would like to thanks Dr Guesmi Salime and Dr Taib Chahinez. Also, I would like to thanks my dear Lamia, Ahlem, Samah, Nadjet,Soumia on the beautiful days we spent together in the labs and the wonderful atmosphere we lived together.

In the end, I would like to thank all those who contributed to the success of this work, and all those who supported me to make it a succes .I would like to thank my dear brothers, each in his name, for standing with me throughout this year and thanking them for their continuous support. I do not forgandmy dear mother, she is a source of my strength and success. I thank her for her prayers to me with success.

Mohcen & Riham

Dedication

I dedicate this work and my gratitude to **my dear parents.**

To my sister **Amira** (and her husband **Arafat**) and 2 brothers **Abdelhafid and Riad.**

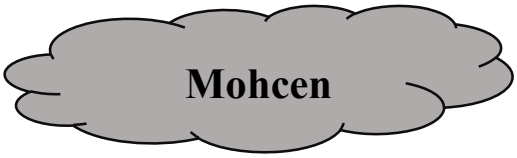
To my big family;**my uncles** in Algiers and Oued-Souf.

To my dear friends **"Aicha Kaouachi, Anfel Djaouadi and Raoinak Melik"** they were there at every stage thanks to the advice and experience they gave me when they obtained their final dissertation.

To all **my teachers** in the school primary to university.

I cannot express my gratitude to them in words.

To all **my friends** who stood next to me and supported me encouraged me throughout the year.



Dedication

I dedicate this memo from the bottom of my heart:

to my mother and my mother and my mother for your love,

the sacrifices you did to make us happy; you have endured a lot of sorrow for my well-being and to my success, receive this by wayratitudeand may Allah keep you a long time and you can trees you planted

to my sister roumaissa for your listening and your support here receive my deep gratitude to your baby anas

to my sister sally and my brother for your affection and family support

to every old and young in my generous family

to my partner of this work mohcen salemi and his family

to my best friend Amira grib

to my every thing in my life

to my partners Roumaissa sid ,Ahlem ,Lamia gharbi and to all those who are near or far, contributed to the realization of this work

without forgetting my dear papa ,my god have mercy on him

Riham brahimi

List of Tables

List of Figures

Abreviations list

µmol Micromoles

½ O2 singlandoxygen

ACh Acetylcholine

AChE Acetylcholinesterase

DNA Deoxyribonucleic acid

DNA Deoxy ribonucleic acid mitochondrial

ADP Adenosine diphosphate

AG Fatty acid

P53 Tumor suppressor protein

RNA Ribonucleic Acid

ATP Adenosine triphosphate

bcl-2 Anti-apoptotic proteins

Ca2 + calcium ion

Cap Caspase

CAT Catalase

SOD Superoxide dismutase

TBA Thiobarbituric acid

TCA Trichloroacetic

SN Nervous system

RE endoplasmic reticulum

RH Oxygenated free radical

LC50 Lethal concentration 50

CO2 carbon dioxide

CPF Chlorpyrifos

GC gas chromatography

Cu Copper

Cyt Cytochrome

ADI Acceptable Daily Intake

LD50 Lethal dose 50

DLM Deltamethrine

EDTA Ethylene-Diamine-Tetraacetic Aci

EOA Activated Oxygenated Species

EPA Environmental Protection Agency

ERN Reactive Nitrogen Species

ERO Reactive Oxygen Species

ESM Standard error on the mean

GABA Gamma-aminobutyric acid

GPx Glutathione peroxidase

Grx Glutaredoxin

GSH Glutathione reduced

GSSG Oxidized Glutathione

GST Glutathione – S-transferase

H hydrogen

H2O2 Hydrogen peroxide

HO Heme Oxygenase

L ° Free lipid radical

LB B lymphocyte

LI learning index

LOO • Radical fatty acid peroxide

LOOH Fatty acid lipoperoxide

LPO Peroxidized Lipids

MA Alzheimer's disease

MAO monoamines oxidases

MAC mitochondrial apoptosis-induced channel

MDA Malon-dialdehyde acid

mgMilligrams

mmolMilimole

mPTtransient mitochondrial permeability

NADPH Nicotinamide-adeninedinucleotide-phosphate reduced

NK natural killer cells

NO Nitric oxide

NOAEL No observed adverse effect level

O2 • Radical superoxide (superoxide anion)

SNC Central Nervous System

Summary

Introduction

Before evolution ,life was simple dependent on agriculture ,as it was a difficult process that used on labor and animals but,that was not enough for the quality of production ,itwas mostly crops spoil due the harmful insects,it causes pathologu for living beigns the protection of agriculture one of the most important operations it often requires a chemical treatment such as pesticides.

Pesticides are products intended to ensure the destruction or prevent the action of animals, plants, micro-organisms or harmful viruses it can be divided into 5 groups: insecticides, nematocidal acaricides, molluscicidal fungicids, redonticids, herbicides, and neonicotinoids **alain viala and alain botta (2005)** Neonicotinoids are the most widely used family of insecticides in the world today. Although the 1st best-selling class of insecticides in the world, with part of the markandexceeding the most important part of sales. In the early 1990 neonicotinoids acquired a large part of the insecticide (**Elbert and *al*., 2008 ; Jeschke and *al*., 2011).**

Ils sont parmis les insecticide les moins persistants dans le sol d'apres **(Hatcher and *al.,* 2008).** The majority of insecticides have a neurotoxic effect on the insect **(Casida.,2009)** neonicotinoids act as nicotinic-type cholinergic receptor agonists, specific to insects vis-à-vis mammals **(Tomizawa and Casida 2003).**

Neonicotinoids are agonists of acetylcholine on postsynaptic nicotinic cholinergic receptors. Their irreversible binding to nAChRs leads to a continuous excitation of the membranes of neurons, leading to cell exhaustion and then to the death of the animal **(Belzunces LP, and *al*., 2012).** The nature of the bonds involved and the chemical structure sites that are known with precision. The different substituents of the molecules of this family are at the origin of a more or less strong affinity for NAChRs. At the intracellular level, phosphorylation mechanisms can also modulate this affinity. Insecticide This is closely linked to activity **(MatsudaK, and *al*., 2001).** Imidacloprid is a neurotoxic insecticide that is part of the neonicotinoid family. It was the first neonicotinoid marketed in the world in 1991. The success of this class of insecticides is due to the irrational persistence of these insecticides in the environment which is accompanied by good efficacy at low dosages, in particular in the field of seed coatings. In addition, these insecticides have a high selectivity towards **insects** compared to mammals (**Tomizawa and Casida, 2003**).The harmful effects and pathologies, social reactions behavior can be corrected by medicinal plants; Many medicinal plants have several therapeutic activities because most often they contain several active principles whose effects are additive and / or complementary. This phenomenon also explains why in

phytotherapy, several plants or parts of plants are frequently used in combination in order to obtain an optimal effect. Among the main plants of our study, the Melissa officialisseknown for its richness in secondary metabolism products and particularly in essential oils and tenin alkaloids **(Baba Aissa, 1999).** The active ingredients in lemon balm are contained in essential oil. Due to its relaxing, sedative and antispasmodic properties, lemon balm is particularly effective in combating stress and anxiety, as well as in treating digestive disorders of nervous origin **(Newman and *al*., 2000)**

We find an extract of lemon balm in the Carmine lemon balm water, which is a reputed alcoholate indicated as antispasmodic, stimulating, for nervous affections, palpitations or even the lack of appetite **(Debuigne, 2013).**herbal teas because of its very pleasant taste, or in hydro-alcoholic extract to benefit from the benefits of its essential oil **(Morel, 2017).** It is found in several drugs **(Vidal, 2018)**

The objective of our experimental work is centered on the evaluation of the subacute toxicity of the new neonicotinoid insecticide "Imidacloprid" and the opposite effect of lemon balm in the oral wistar rat. To this end, we studied ourselves studying its impact on body weight on certain biochemical parameters as well as on the essential organ the brain . Confirmation of the optimal correction of Melissa is done by analyzing biochemical , oxidative stressneurobehavioural and their implication on the structural integrity of brain cells and we evaluated the preventive effects of Melissa from the results obtained , we divided the work into 2 main parts, one theoretical and the other practical we will look at them in the content

First part

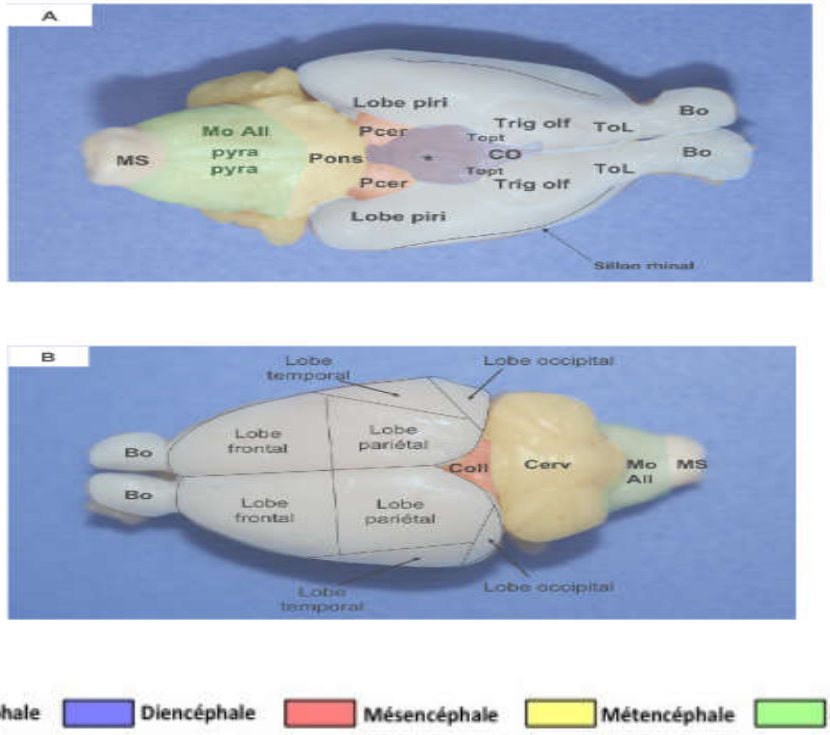
Bibliographic

Chapter 01 :

Nervous system

1. The nervous system:

Nerve tissue is made up of neurons and neuroglia. The latter has a supporting role, it is a filling tissue that proliferates and can fill the foci of destruction of nervous tissue. It would also have a nutritive role and perhaps a role in the conduction of nerve impulses.The entire nervous system is made up of multiple chains of neurons: in a chain the connection between the neurons is made so that the cylindrax of one comes into play with the dentrite or pericaryon of the other. There is never a cylinder-to-cylinder or dentrite-to-dentrite connection.The point where the cylindrax connects with the dentrites of the following neurons is called a synapse. These are the inter-neural connections.**(Georges and *al.*,2008)(Figure 02).**



(A), (B): Bo: olfactory bulb, Cerv: cerebellum, CO: optic chiasma, Coll: collicles, Piri lobe: piriform lobe, Mo All: elongated marrow, MS: spinal cord, Pcer: cerebral peduncle, pyra: pyramid, ToL: olfactory tract, T opt: optic tract, Trig olf: olfactory trigone, *: infundibular recess of the 3rd ventricle.

Figure01: Ventral (A) and dorsal (B) views of a rat brain (**http://oatao.univ-toulouse.fr/**)

1.1. Brain histology:

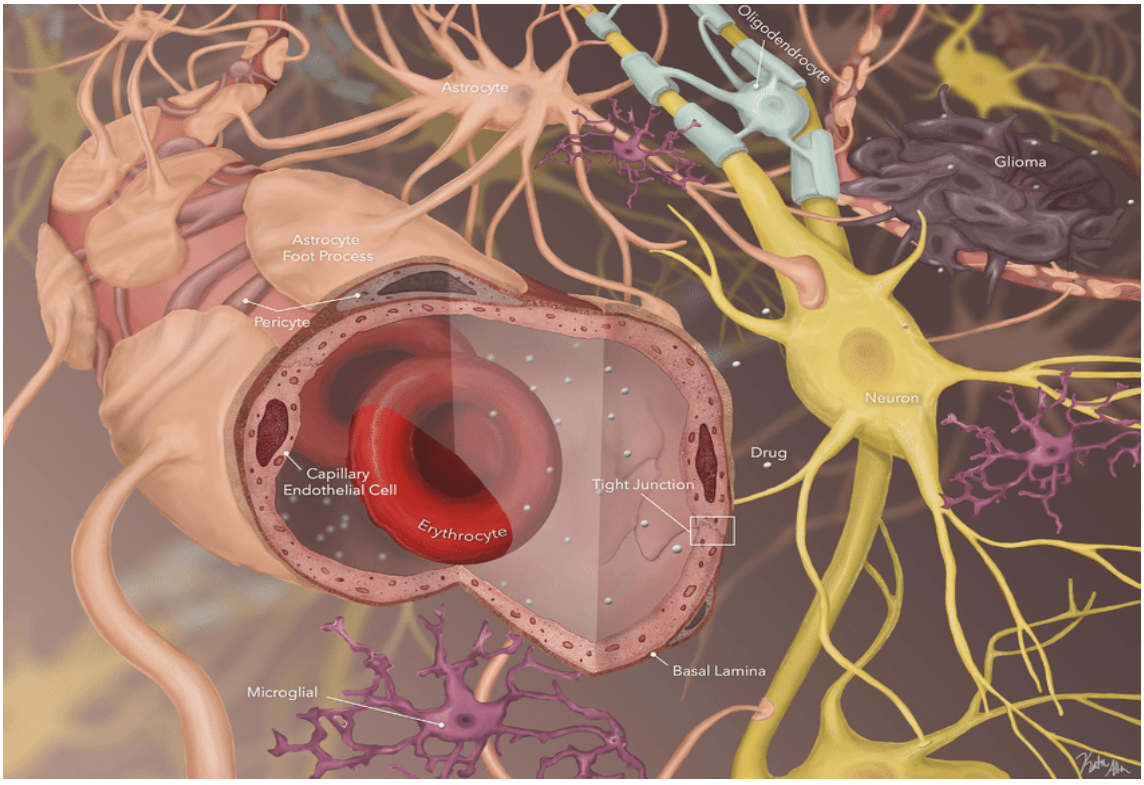
The nervous system (NS) is the sandof systems that put the body in a state of equilibrium. It is subdivided into two systems: central (CNS) and peripheral (PNS).

- The CNS: It is the center of regulation and communication of the organism, it is formed of the brain and the spinal cord **(Bear and *al.*, 2016).**
- The PNS : it is the communication line between CNS and the organization ,is located at the end of CNS it consists mainly of nerves (cranial and spinal **).** From the brain and spinal cord which includes two types of pathways , **(Marieb, 2005).**

1.2 brain physiology:

The NS detects the various stimulations (external and internal) and reacts quickly with a very high coordination, the electrical signals consist of action potentials which propagate quickly along the axons from one neuron to another or from one neuron to an effector cell **(Imbert, 2006; Verkhratsky & Butt, 2007; Bear and *al.*, 2016).**

- Neurons:



A neuron is a fundamental NS cell specializing in information processing and communication. The neurons which carry information coming from the sensory receptors of the organism, they are composed of the cellular body surrounded by dendrites and axon finished by arborization of the synaptic buttons **(Figure02) (William 1981; Pascal 2010).**

Figure02: Schematic representation of the brain cell parenchyma.

Neurons are surrounded by glial cells, among which astrocytes are a privileged partner in the regulation of neuronal function.

The main role of neurons is the transmission of the nerve signal;it is the glial cells that are responsible for protecting them, nourishing them and optimizing their functioning **(Christensen and *al*., 2013).**

- **Glial cells:**

There are four types of glial cells in the NS; oligodendrocytes, microglia, ependymaries and astrocytes **(William, 1981; Block and Hong, 2005**). They surround neurons and participate in controlling the chemical and electrical environment by providing them with nutrients and eliminating their waste **(William, 1981).** They are called neuronal support cells because they produce growth factors and reuse neurotransmitters **(Figure03) (Christensen and *al*., 2013**). Glial cells present a very important role in the pathology of the nervous system. **(Koistinaho and *al*., 2004; Pihlaja and *al*., 2011).**

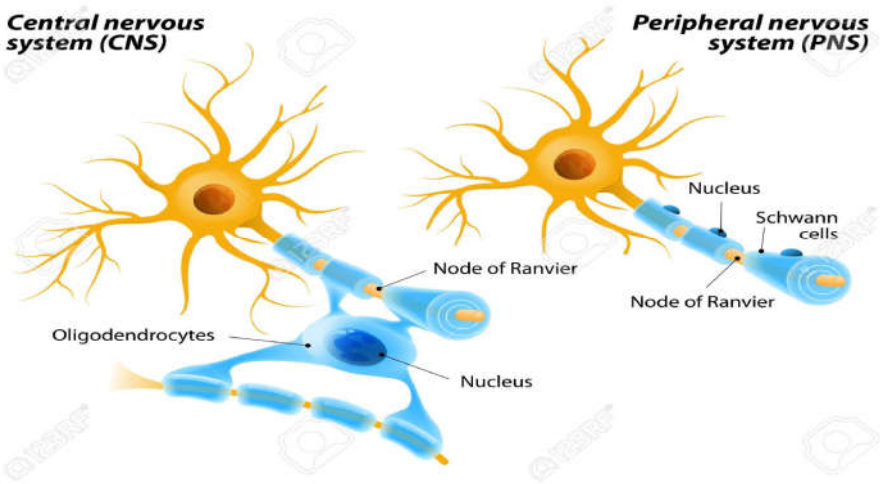


Figure03: Structures and shapes of glial cells (**https://www.123rf.com**)

In addition, astrocytes are considered to be a major source of peroxynitrite production, the latter inducing the nitration of proteins including amyloid β in Alzheimer's disease, , interfere with several signaling pathways, disrupt mitochondrial respiration and cause cell death **(Kummer and *al.*, 2011).**

2. Functioning of the nervous system:

2.1 Nerve flow and synaptic integration:

The transmission of nerve impulses by nerve cells is due to the electrochemical properties of their plasma membranes. The signal is transmitted from one nerve cell to another

through junctions called synapses **(Purves and *al*., 2004; Brooker and *al*., 2001),** a very specific type of these synapses is that which connects the neuron to an effector cell such as a muscle fiber, this synapse is then called neuromuscular junction **(Gerard and *al.*, 2002; Ganong and *al*., 2005).** The passage of information in a synapse is ensured by chemical molecules called neurotransmitters **(Kolb and *al.*, 2002).** At the synapses, when the nerve impulse reaches the presynaptic terminations trigger events which lead to the release of a neurotransmitter through the synaptic cleft which modifies the permeability of the postsynaptic membran **(Marieb, 2005).** The Na + voltage dependent channels are responsible for the initiation and propagation of these action potentials along the,selective axon for ions Na+. at rest channels are closed when the membrane is polarizes, these channels are open **(Gueguen and *al.*, 2005 ; Imbert, 2006).**

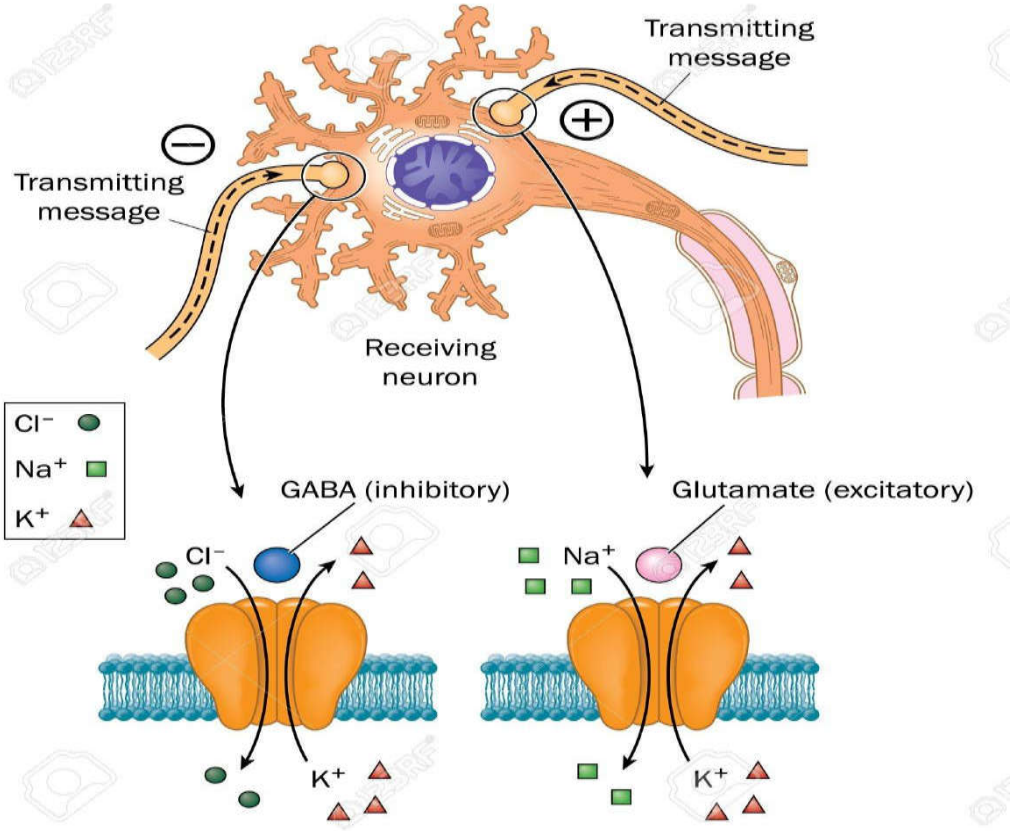


Figure04: Mode of transmission of nerve signals (**https://fr.123rf.com**)

The ability of a neuron to accept and relay information results primarily from differences in the distribution of ions on the cell membrane **(Bear and *al.*, 2016).** This creates a difference in electrical potential between the inside and the outside of the cell at rest **(Lacombe, 2006),** and secondly, momentary modifications in the permeability of this membrane to certain ions, which generates an electrical depolarization of the membrane. The latter is transmitted step by step along the axon and constitutes the transmission of nerve

information or nerve impulse **(Siegel and *al.,* 2005).** After being depolarized, the membrane quickly returns to its initial polarized state, but until the ions have returned to their place, the axon cannot conduct new influx, this period is called refractory **(Figure05) (Cambier, 2008; Lacombe, 2006).**

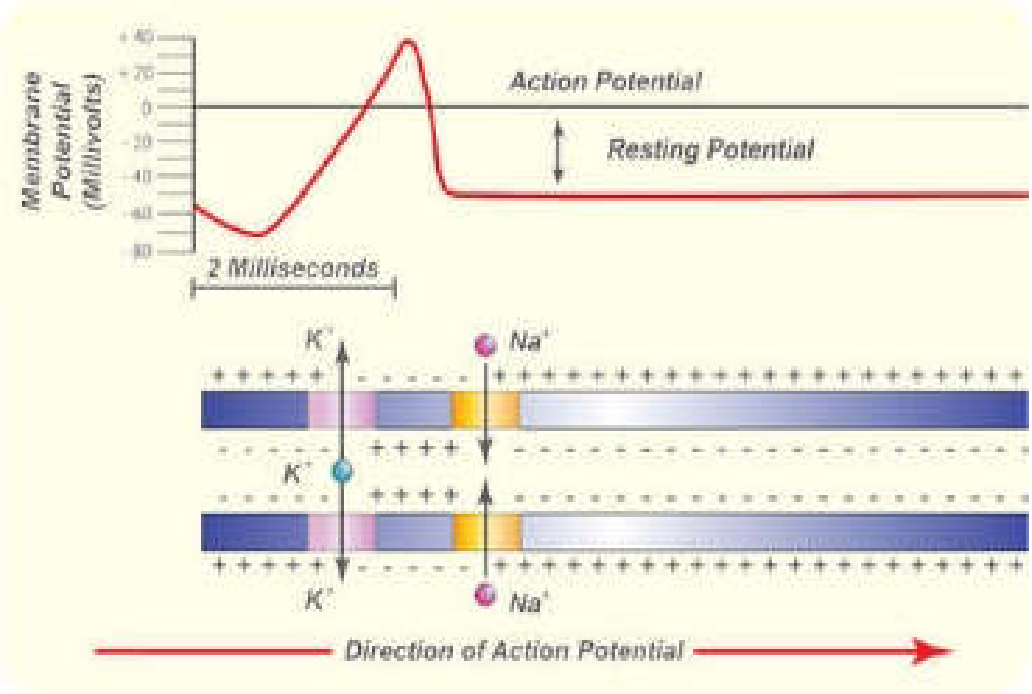


Figure05: Variation of the electrical potential of the axon cytoplasm compared to the outside, and origin of depolarization (**https://www.ck12.org**)

In amyelin fibers, the speed is only about 1.5-2 m / s, while it reaches 100 m / s along the myelin fibers **(Cambier, 2008),** In the latter case, the electrical depolarization moves by jumps, from Ranvier node to Ranvier node, the myelin sheath being electrically insulating. . The first myelin sheaths appear only at the end of fetal life and during the first year, and the amount of myelin increases until maturity, it is easy to understand why the reactions of a child and especially of a newborn baby are not so fast and as coordinated as those of an adult **(Figure06) (Braillon, 2002; Gueguen and *al.*,2005; Bernard and *al.*, 2007).**

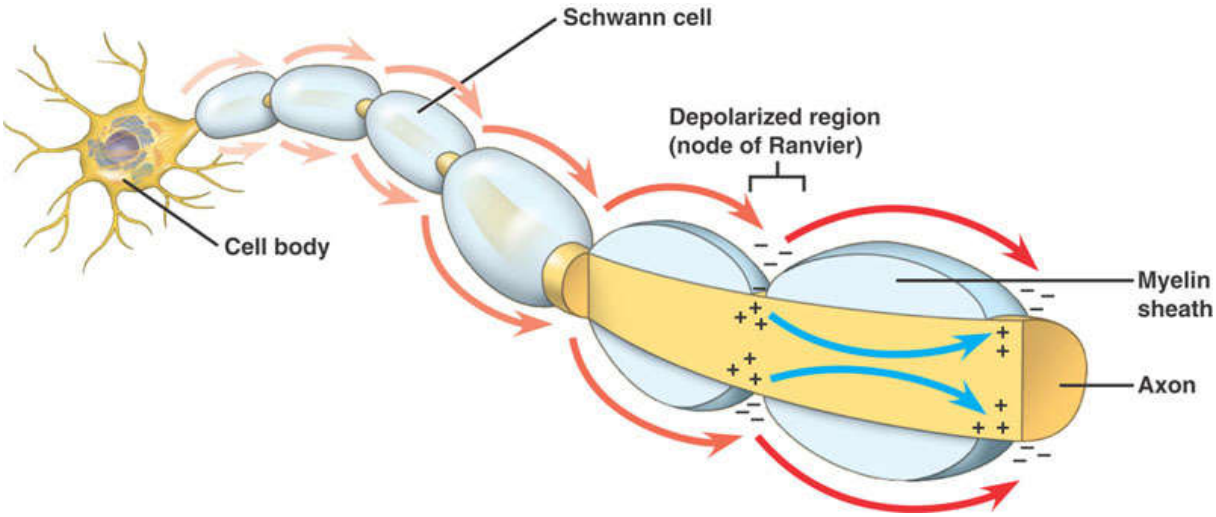


Figure06: Presentation of the progression of nerve impulses along an amyelin neuron (top) and along a myelin neuron (bottom) **(https://www.afblum.be/bioafb)**

The nerve impulse must frequently be transmitted from one neuron to another: it does so at the level of a synapse or synaptic junction **(Cambier, 2008; Lacombe, 2006).** In the terminal buttons there are small synaptic vesicles containing neurotransmitters or neuromediators, substances synthesized by the neuron **(Figure07) (Tritsch and *al.*, 1999).** When the nerve impulse reaches the synaptic button, it causes the release of neurotransmitters in an intimate space of 20 to 50nm (synaptic cleft) separating the pre and postsynaptic neurons **(Cambier, 2008).** These neurotransmitters bind to receptors on the membrane of the postsynaptic neuron and induce, depending on their chemical nature, several reactions **(Tritsch and *al.*, 1999).**

2.2 Neurotransmitters:

The chemical transmission of nerve information at the synapse imposes a transmission delay of 0.5 ms, whereas it would only be 1.10 to 6 s in the case of a purely electrical transmission. But the chemical nature of this mode of transmission makes it possible to act on the propagation of the nerve impulse **(Verkhratsky & Butt, 2007).**

Neurotransmitters are chemicals released by a neuron at a synapse that specifically changes the activity of another cell. They diffuse to the post-synaptic region to activate their receptors then are quickly eliminated **(Guenard, 2001)** either by diffusion outside the synaptic cleft, either degraded by a specific enzym and reabsorbed by the terminal button **(Gueguen and *al.*, 2005).**

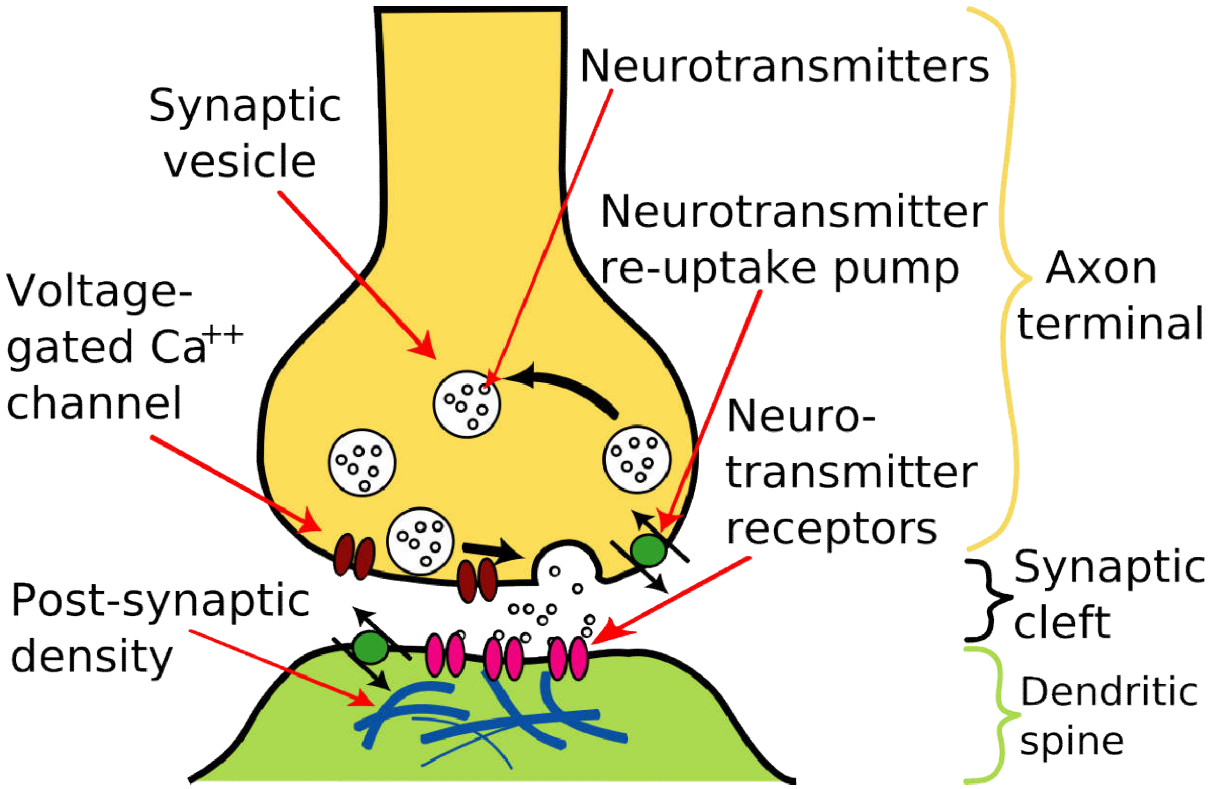


Figure07: Mechanism of synaptic transmission of nerve impulses (**https://www.bio-top.net**)

- Acetylcholine (Ach):

Once at the nerve end, the motor impulse causes the calcium channels to open, triggering a massive entry of calcium ions into the cell. Ca2 + promotes the secretion of Ach in the synaptic cleft **(Guenard, 2001; Kolb and *al.*, 2002).** Each two Ach molecules diffuse on the other side and linked with a cholinergic receptor **(Lodish, and *al.*, 2005; Martin and *al.*, 2006),** the latter causes the opening of a sodium channel, which favors the entry of sodium ions inside the muscle fiber, , thereby depolarizing the postsynaptic membrane and creating plaque potential **(Lodish, and *al.*, 2005).** Depending on the number of activated receptors, this potential can exceed a threshold value and thus trigger a muscle action potential which will diffuse to the entire muscle membrane. and cause a contraction of the muscle fiber **(Figure07).** The Ach molecules are quickly destroyed by the AchE enzyme present at the two molecule synaptic cleft; acetate and choline **(Martin and *al.*, 2006).**

- Adrenaline (Ad):

Adrenaline plays an important role in regulating all organs and the body's reaction to stress, in the event of critical or harmful situations (danger, trauma, infection, cooling). The release of this neuromediator then involves a whole series of involuntary nervous reactions,

such as the acceleration of the cardiac and respiratory rhythms to prepare the body for the effort of the fight or the flight **(Martin and *al.,* 2006; Verkhratsky & Butt, 2007).**

- Dopamine (Da):

Dopamine is a neurotransmitter synthesized by certain nerve cells from tyrosine **(Bear and *al.,* 2016**). It is the inhibitory neuromediator of a small group of neurons that affects muscle movement, tissue growth, and the functioning of the immune system. It is involved in the secretion of growth hormone **(Kazushige and *al.,* 2000; Meiser and *al.,* 2013; Dias and *al.,* 2014).**

- Gamma-Aminobutyric acid:

GABA or g-aminobutyric acid is an inhibitory neurotransmitter, the receptor of which also binds to valium. GABA and valium help relieve anxiety and decrease anxiety. It is the main inhibitory neurotransmitter and it plays a critical role in the regulation of neuronal activity (membrane hyperpolarization) in vertebrates and invertebrates **(Buckingham and *al.,* 2005; Verkhratsky & Butt, 2007)**

- Serotonin (5-HT):

Serotonin or 5-hydroxytryptamine (5-HT) is an inhibitory neurotransmitter thought to intervene in phenomena such as sleep, consciousness and emotional states **(Wang and *al.,* 2007).** The serotonergic system involves 5-HT which is considered to be the most important neurotransmitter involved in anxiety. An anxiolytic acting via the cerebral serotonergic system has been shown to decrease the level of 5-HT in the brain **(Hoyer and *al.,* 2002; Caramaschi and *al*., 2007; Meneses and *al.,* 2007).**

- Morphine:

The natural morphines of the human body are neuropeptides called endorphins and enkephalins **(Martin and *al.,* 2006).** Enkephalins are inhibitors and competitors that are found in synapses treating the influx of mood, emotion and pain: by attaching to post synaptic receptors in place of effector neurotransmitters, they block the propagation of nerve impulses **(Gueguen and *al.,* 2005; Nieuwenhuys and *al.,* 2008).**

3. Major neuronal pathologies:

3.1. Alzheimer's disease:

Alzheimer's disease is a neurodegenerative disorder that causes permanent and irreversible progressive damage to nerve cells, which begins with the loss of memory of recent events and then the loss of cognitive functions (aphasia, apraxia, agnosia) **(Kelley, 2011; Wang and *al.*, 2016; Bonda and *al.*, 2015).**

3.2. Parkinson disease:

A neurodegenerative disease that results from the slow and progressive death of neurons in an area of the brain that plays an important role in controlling our movements. This is why those affected gradually make rigid, jerky and uncontrollable gestures **(Defebvre and Verin, 2006; Verkhratsky & Butt, 2007).**

3.3. Brain tumor:

Multiplication in the brain of abnormal cells . The tumor can be benign or malignant **(Peterson and *al.*, 2015).**

Chapter 02 :
Neonicotinoids

1. Definition:

Neonicotinoids are synthetic analogs of nicotine with insecticidal properties **(Table 1)**. Introduced on the markandbetween 1991 and 2002, they were notably created to thwart the resistance of insect pests against previous classes of insecticides (organophosphates, carbamates and pyrethroids). In just two decades , they have managed to appropriate 23,7%of world sales of insecticides ,thus rising to the top of this markand. Currently approved in over 120 countries for phytosanitary and veterinary purposes, neonicotinoids are mainly used in agriculture. often prophylactically, on corn, soy, canola, cereal, cotton and sugar beandcrops. Phytosanitary interventions are done by soil treatment, foliar application and coating of seeds. Veterinary treatments, for their part, include the oral administration of tablets or the application of topical products6 (liquids and collars).

The most widely used and available neonicotinoid insecticides are imidacloprid, thiamethoxam, clothianidin, nitompyran, acetamiprid, dinotefuran, thiacloprid **(Jeschke and *al.*, 2011) (Table01)**

Table 01: Different pesticides belonging to the neonicotinoid family: **(Jeschke and *al.*, 2011)**

Physical property	Synonyms	Chemical substances
Solid white	Acetamiprid (E) -N - [(6-Chloro-3 pyridinyl) methyl] N'-cyano N-methylethanimidamid	Acetamiprid C10H11ClN
Colorless solid	Clothianidin 3-[(2-chloro1,3-thiazol-5-yl)methyl]2methyl-1-nitro-guanidine	Clothianidin C6H8ClN5O2S
Solid white	Dinotefuran 2-methyl-1nitro-3-(tetrahydrofuran3ylmethyl)guanidine	Clothianidin C6H8ClN5O2S
Solid colorless or beige	Imidacloprid 1- (6-chloro-3pyridylmethyl) Nnitroimidazolidin-2ylideneamine	Imidacloprid (2 isomers) C9H10ClN5O2
Solid jaunt Clear	Nitenpyram (E) -N - [(6chloropyridin-3-yl) methyl] Nethyl-N'-methyl-2nitroethene-1,1diamine	Nitpyrame C11H15ClN4O2
Solid	Thiacloprid (Z) -3- (6-chloro3-pyridylmethyl) 1,3thiazolidin-2ylidenecyanamide	Thiacloprid C10H9ClN4S
Solid	Thiamethoxam (EZ) -3- (2chloro-1,3-thiazol5ylmethyl) -5-methyl-1,3,5oxadiazinan4ylidene (nitro) amine	Thiamethoxam C8H10ClN5O3S

2. Terms of use:

The arrival of neonicotinoids was a commercial success following years of research. Today they are widely used in agricultural and other non-agricultural uses.

2.1. Historical:

The first molecules belonging to the neonicotinoid family were synthesized in the 1970s. The term "neonicotinoid" was proposed by the Japanese researcher Izuru Yamamoto to differentiate this family from the old "nicotinoids", that is, nicotine-containing plants, used as insecticides since the 18th century **(Jeschke, Nauen 2008; Matsuda and *al.* 2001).**

The insecticidal power of the first molecules tested was however very low, research was therefore continued to identify the active chemical groups and synthesize molecules with better activity. Nithiazine was one of the first molecules of interest with satisfactory insecticidal activity, systemic distribution in plants, and low toxicity in vertebrates. But it was quickly degraded by hydrolysis or photolysis, making it impossible for agricultural use. About 2,000 molecules were tested before the discovery of imidacloprid by Shinzo Kagabu and its introduction to the markandin 1991 by Bayer CropScience. The family then grew and now has seven compounds on the market: imidacloprid and thiacloprid (developed by Bayer CropScience), clothianidin (Bayer CropScience and Sumitomo), thiamethoxam (Syngenta), acetamiprid (Nippon Soda), nitenpyram (Sumitomo), and dinotefuran (Mitsui Chemicals). An eighth compound, sulfoxaflor, has recently been placed on the markandin China and the United States and has been examined by the European Food Safety Authority (EFSA) for approval in the European Union. In China, new neonicotinoid compounds are being developed and tested (for example, guadipyr and huanyanglin) and are close to being put on the market. **(Jeschke and *al.,* 2011; Simon-Delso and *al.,* 2015)**

The arrival of neonicotinoids on the insecticid pesticid markandwas an immediate success for several reasons **(Jeschke, Nauen 2008; Simon-Delso and *al.,* 2015):**

- There was no known resistance to these pesticides in pests, unlike organophosphates, carbamates and pyrethroids.
- Their physicochemical properties made them more interesting compared to previous generations of insecticides: they are active in much lower quantities than other families, their use in coating seeds rather than in spraying limits waste, and they have a systemic distribution.

- They have low toxicity for the user and non-targandspecies (other than arthropods) thanks to their very selective mode of action of insects.

They have thus become the most widely used insecticides among the 5 major chemical classes, ahead of organophosphates, carbamates, phenylpyrazoles and pyrethroids. Neonicotinoids have found applications in agricultural environments, in amateur gardens and in domestic use to combat certain pests, as well as in the veterinary field with the control of external parasites of domestic animals **(Jeschke and *al.*, 2011).**

2.2. Use:

2.2.1. Obtaining RA:

For any marketing of a new neonicotinoid product, the manufacturer must obtain a regulatory affairs (RA).

To do so, he must submit a file demonstrating:

- cultural efficiency
- safety for humans
- harmless to animals and the environment
- compliance with national and European legislation

2.2.2. Agricultural use:

2.2.2.1. Interests:

Neonicotinoids are the most widely used family of insecticides in the world today. Use as an agricultural insecticide is the largest share of sales. They are used in spraying and coating seeds for their insecticidal effects against many pests, even resistant to other families of insecticides, such as aphids, thrips, termites, whiteflies, wireworms and certain beetles. Their use spread very quickly throughout the world on rice crops, cereals, corn, sunflower, potatoes, cotton, sugar beandand on fruit trees and vegetable crops. The methods of use are varied: coated seeds, soil treatment and spraying mainly, but also application to the base of tree trunks, injection of tree trunks and buds, or even soaking and chemo-irrigation for greenhouse crops. The use is not only professional, neonicotinoids are also found in products for household use for the fight against certain pests such as termites, cockroaches or ants **(Casida, 2004; Jeschke and *al.*, 2011; Bonmatin and *al.*, 2015; Goulson 2013).**

2.2.2.2. Some figures:

Neonicotinoids are authorized in more than 120 countries worldwide. in 2008 they representes a markandof 1,7 billion euros ;or a quarter of all insecticides ,in front of organophosphorus (13%) and carbamates (11%) , They largely dominate the coated seed market: at the time of their marketing, the coated seeds represented 155 million euros in total and in 2008 this amount increased to 957 million euros, 80% represented by neonicotinoids **.**In the same year, **imidacloprid** became the world's best-selling insecticide and the second-largest pesticid sales (glyphosate being the best-selling). Among neonicotinoids, **imidacloprid** is the major representative, the world's best-selling insecticide, accounting for 42% of neonicotinoid sales, ahead of thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran and finally nitempyram**. (Nauen, 2008; Girolami and al. 2009; Jeschke and *al.,* 2011; Sánchez-Bayo, Hyne 2014; Simon-Delso and *al.,* 2015)**

2.2.3. Veterinary use:

Neonicotinoids are rapid insecticides with very selective effects: their use in domestic animals is interesting. The (RA) concern flea infestations in cats, dogs and rabbits. The spectrum of action can be supplemented by a pyrethroid to act also against ticks, mosquitoes, sand flies and biting flies in dogs and cats, Finally, an association with moxidectin widens the spectrum to internal parasites of cats, dogs and ferrets**. (ANSES 2016; Hovda, Hooser 2002; Jeschke and *al.,* 2011).**

3. Physico-Chemical Characteristics:

Their chemical structure is at the origin of their common properties. These physical characteristics condition their fate in the environment and determine their selective mode of action by Insects.

3.1. Chemical structure:

The structure of neonicotinoids is divided into 3 segments (figure11) **(Iwasaand *al.,* 2004; Jeschke, Nauen 2008; Jeschke and al. *al.,* 2011)**

I- A group "R1" and"R2" cyclic (imidacloprid, thiamethoxam, thiacloprid) or acyclic (acetamiprid, nitenpyram, clothianidin, dinotefuran).

II- An "A" ring of 5 or 6 atoms, with one chlorine atom.

III- A cyano or nitro functional group "[X-Y]", essential for insecticide activity.

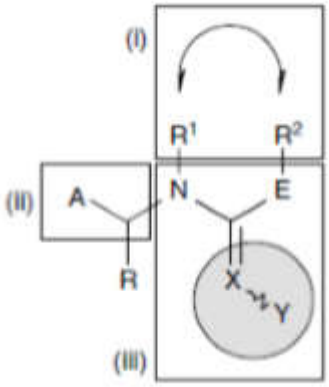


Figure08: Structure of neonicotinoids **(Jeschke, Nauen 2008)**

Depending on this functional group ,we can classify molecules according to common physical properties **(figure8) (Jeschke, Nauen 2008) :**

- **Nitrosamines:** thiamethoxam, dinotefuran dithiazine, imidacloprid, clothianidin and nitenpyram.
- **Cyanoimines:** thiaclprid and acetamiprid.

Insecticidal power is also correlated with chemical structure (Iwasa andal. 2004; Decourtye, Devillers 2010; Matsudaand al. 2001):.

- Molecules with an “A” ring with 5 carbon atoms are less powerful than those with a 6 atom ring.
- The presence of oxygen, sulfide or nitrogen substituents increase the insecticidal action.
- The substituted nitro molecules have a better insecticidal action than the cy.anosubstituted.

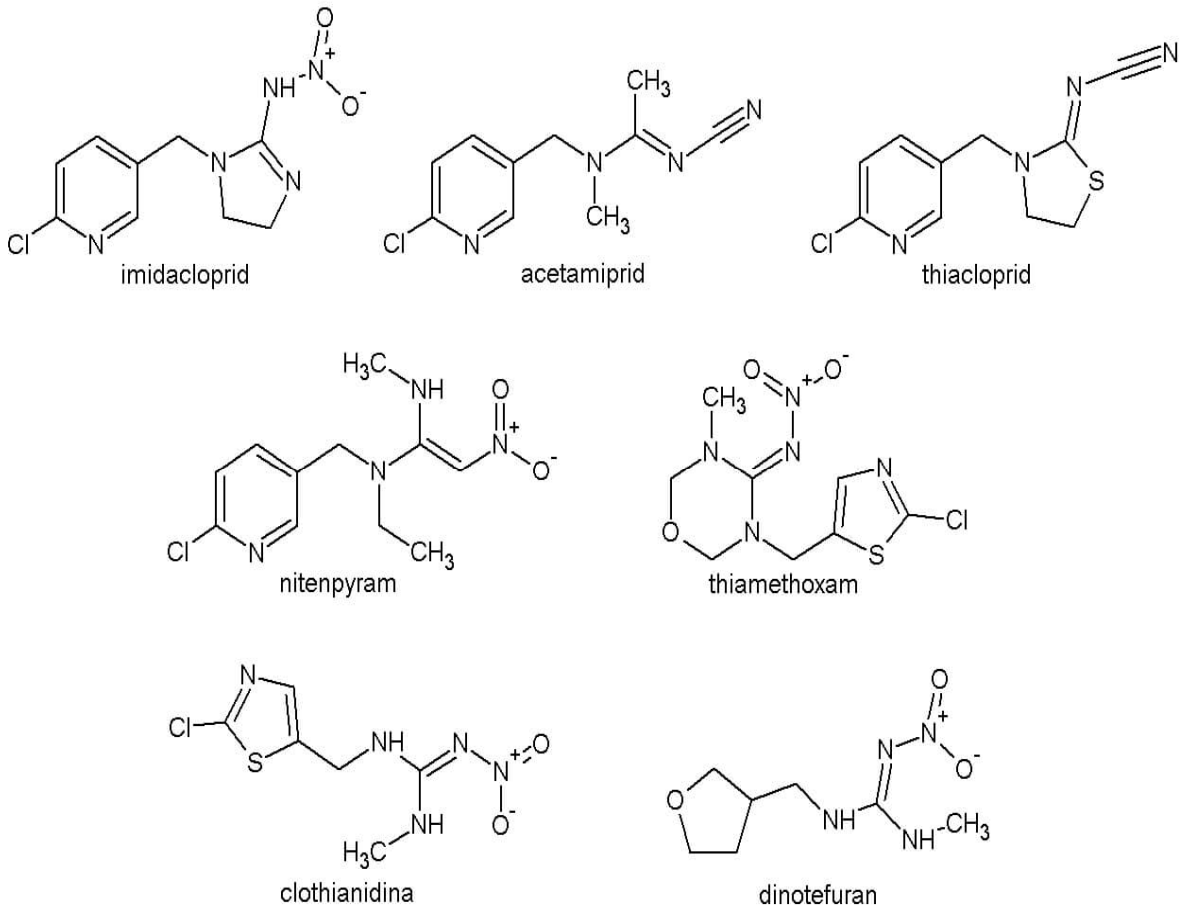


Figure09: Flat formulas of the main neonicotinoids**.(Simon and *al.*,2015)**

3.2.Physical properties:

3.2.1. Molecular weight:

The molecular weight of neonicotinoids is between 250 and 300g / mol, making them **low-weight insecticides**: for comparison, the weight of fipronil, another systemic insecticide, is 440g /mol. **(Bonmatin and *al.*, 2015)**

3.2.2.Volatility:

Neonicotinoids have a saturated vapor pressure between 2.8.10-8 and 0.002 mPa at 25 ° C. This makes them little volatile molecules, they contaminate the air little during the treatments, and airborne contamination lasts shortly after spraying. **(Bonmatin and *al.*, 2015; Fossen 2006)**

3.2.3. Sorption:

Sorption characterizes the ability to bind to particles. Neonicotinoids can bind to soil particles, limiting the leaching phenomenon that causes water contamination. Several factors come into play: the organic matter content as well as the clay content are positively

correlatedwith the sorption of imidacloprid, while a low temperature or low insecticide concentration decreases sorption. Neonicotinoids also attach to particles in bottom sediments of freshwater or marine water **(Bonmatin and *al.*, 2015; Fossen 2006; Gervais and *al.*, 2010).**

However, in commercial preparations, the addition of many substances changes the behavior of the active substance: so leaching is higher with all commercial formulations compared to imidacloprid alone **(Bonmatin and *al.*, 2015)**

3.2.4: Solubility:

The solubility in water varies according to the molecules, from 184mg / L for thiacloprid (moderate solubility) to 590g / L for nitenpyram (high solubility). These values are valid at 20 ° C for a pH of 7. Compared to other insecticide families, neonicotinoids have good solubility: for example, under the same conditions, the solubility of fipronil is between 1.90 and 3.78 mg / L**. (Jeschke, Nauen 2008; Bonmatin and *al.*, 2015)**

The variations in solubility are correlated with their structure:

- Open compounds are more hydrophilic than cyclic compounds
- for the group [X-Y], the solubility in water increases with increasing [= N-NO2] <[= N-CN] <[= CH-NO2]

Solubility is important since it conditions their absorption then their diffusion, essential steps for their effectiveness. For example the least water-soluble neonicotinoids such as thiacloprid or clothianidin will be suitable for seed treatment, the membrane passage in the roots being better for lipophilic compounds **(Jeschke, Nauen 2008; Jeschke and *al.*, 2011).**

4. Metabolism:

Like all molecules toxic to a given organism, neonicotinoids are metabolized by the organism into which they have introduced. Although it is difficult to know all of the metabolites that result from it, some of the main metabolites are known. The work of **(Suchail and *al.*, 2003)** clearly determined the metabolites of imidacloprid, an insecticide that belongs to this family.

The different metabolites of imidacloprid are: 5-hydroxy-imidacloprid (5.OH), 4,5-dihydroxy-imidacloprid (4,5-OH), olefin, G-chloronicotinic acid 6-ACN) and guanidine and urea derivatives **(Figure 10)**

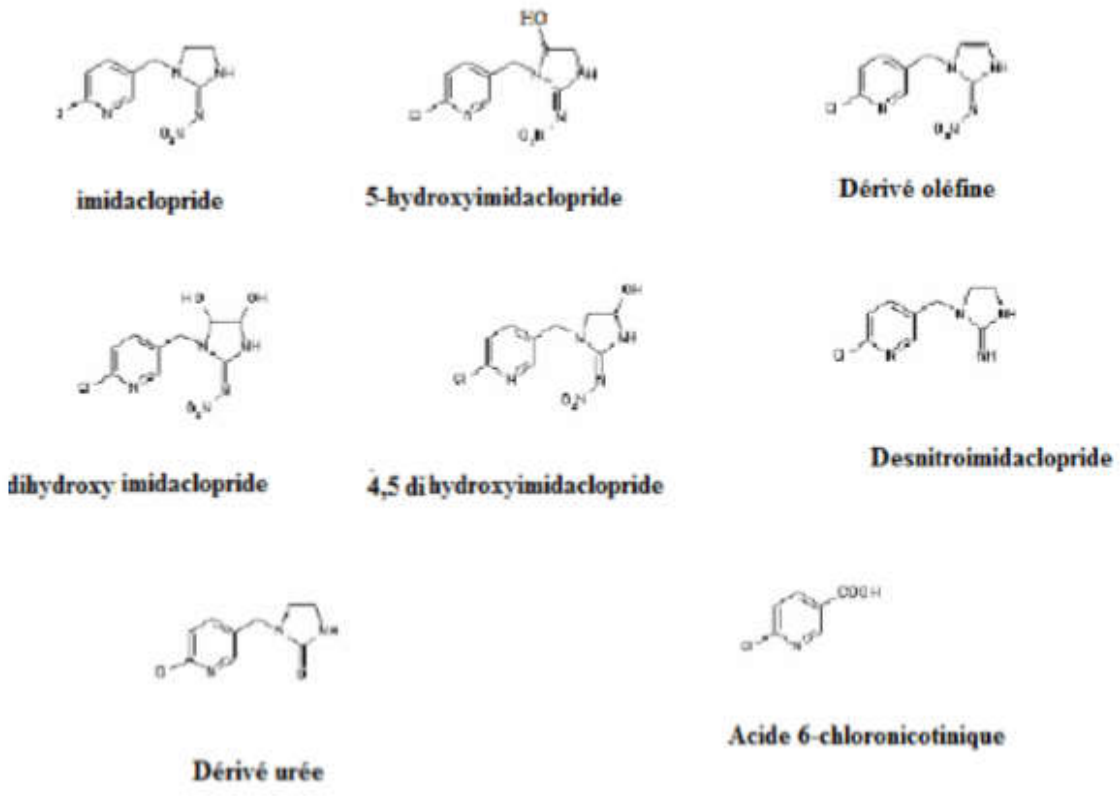


Figure10: main metabolites of imidacloprid **(Suchail and *al.,* 2003).**

5. Mode of action:

The nervous system is made up of a network of neurons interconnected through specific junctions: synapses. The message conveyed at a synapse can be electrical or chemical (neurotransmitters). However, regardless of the nature of the signal, the perception of information in the post-synaptic cell causes activation or the inhibition of receptors or membrane ion channels **(Raymond-Delpech and *al.,* 2005).**

Acetylcholine is the main excitatory neurotransmitter for rapid transmissions in the central nervous system. During nerve impulses, acetylcholine is released by the presynaptic membrane and will interact with the nicotinic acetylcholine receptor (AChR). This receptor forms an ion channel whose opening depends on the fixation of acetylcholine, resulting in an extracellular Na+ influx and an intracellular K+ influx, thereby triggering the nerve impulse. The breakdown of acetylcholine by acetylcholinesterase stop the signal. Nicotine is a non-hydrolyzable acetylcholine agonist, it remains attached to the receptor which prevents it from closing, and which disturbs the signal by creating hyperpolarization of the cell **(Arreola and *al.,* 2011; Jeschke and *al.,* 2013)**

). Nicotinic receptors are composed of five subunits which form a channel allowing the selective passage of Na +, Ca2 +, and K + ions **(Efsa, 2014).** Neonicotinoids, neurotoxic

insecticides, act specifically on acetylcholine receptors (nACHRs) as a competitive inhibitor, that is, it blocks the transmission of impulses from the synaptic membranes of the nervous systems **(Figure11).**

This dysfunction eventually causes tetany (muscle contracture crises) **(Buckingham and *al.*, 1997).** The majority of its insecticides are dual action, act by contact and ingestion at very low doses **(Matsuda and *al.*, 2001; Nauen 2006; He and *al.*, 2012)**

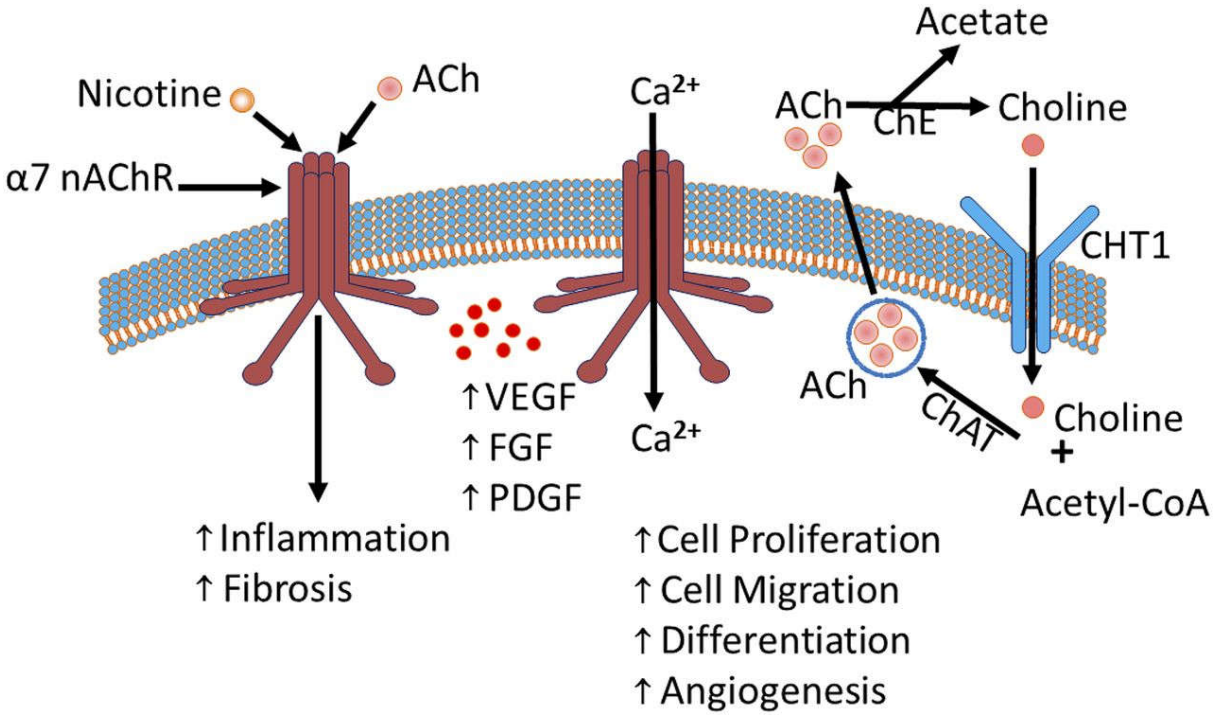


Figure11: Schematic representation of a cholinergic synapse between two neurons with the main targets of neonicotenoids. ACh: acetylcholine; AChE: acetylcholinesterase; nAChR: nicotinic type cholinergic receptor; Na: sodium channel **(Raymond-Delpech and *al.*, 2005).**

6. Carcinogenic effects:

Acute intoxication usually occurs immediately or shortly after exposure to a pesticide. The most frequent symptomatic signs encountered during acute poisoning are headache, nausea, vomiting, dizziness, fatigue, loss of appetite andand skin or eye irritation, difficulty breathing, seizures and even coma **(Samuel, 2012).** These harmful effects can be reversible or irreversible **(Engel and *al.*, 2005)**. However, chronic intoxication generally occurs following the repeated absorption of low doses of pesticides over a long period. The main effects of chronic exposure to pesticides are effects on reproduction, development, the immune and endocrine systems as well as carcinogenic effects **(Samuel, 2012).**

6.1. The carcinogenic effects of neonicotinoids on the thyroid gland:

The thyroid is an endocrine gland located on the anterior side of the neck and regulating, in vertebrates, many hormonal systems by the secretion of triiodothyronine (T3), thyroxine (T4) and calcitonin. T3 and T4 bind iodine to a protein, under the influence of TSH, a hormone that regulates the functioning of this gland **(Reynolds and *al.*, 2002).** In the thyroid there are many small, rounded, bag-like structures called follicles. The follicles produce, store and release thyroid hormones. The thyroid is made up of different types of cells. The follicles are lined with follicular cells, C cells (also called parafollicular cells) which are scattered throughout the thyroid, including between the follicles and in their coating. Among the other thyroid cells,there are lymphocytes (a type of white blood cell) and fat cells (called adipocytes) **(Reynolds and *al.*, 2002).(Figure 12)**

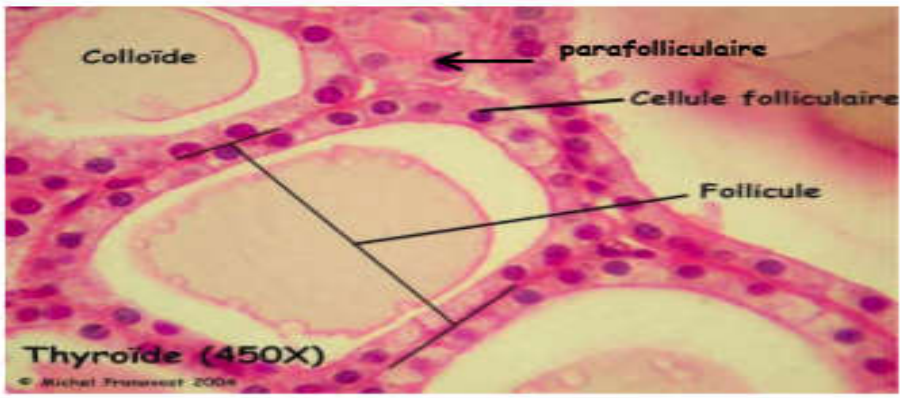


Figure12: histology of thyroïde 450x (http://cancerthyroide.blogspot.com).

The impact of neonicotinoids on this gland has been the subject of several studies. Oral administration of thiamethoxam has caused an enlarged thyroid gland in rabbits **(Shalaby and *al.*, 2010). (Nicolle-Mir,2011)** have shown that exposure of male rats to imidacloprid results in a significant increase in the absolute mass of the thyroid **(Tebourbi and *al.*, 2010)** reported that injecting another compound, acetamiprid, into the rats caused a significant increase in the relative weight of the thyroid gland. However, atrophy of the thyroid gland, manifested by a decrease in its absolute mass, has been reported after treatment of rats with other neonicotinoids, fipronil and acetamiprid **(Surks and *al.*, 2004) (Johnson and *al.*, 2009).** The impact of neonicotinoids in rats remains unclear. The work of Saadi and al., (2014) showed the toxicity of a neonicotinoid imidacloprid in wistar rats, after administration of two doses at 3.67 mg / kg / day and 5.18 mg / kg / day for 30 days orally. The results obtained revealed the quantitative assay of the thyroid hormones FT3 and FT4 (measured by the ELISA technique).

The results obtained shown in Table 02 do not reveal any significant deference in FT3 and FT4 between the control series and the treated series.

Table02 : Effect of a neonicotinoid elidacloprid administered at two doses 3.67 and 5.18 mg / kg / day on the thyroid hormones FT3 and FT4 in the wistar rat **(Saadi and *al.*, 2014).**

Groups	FT3 (mol/l)	FT4 (mol/l)
Control	6.81± 1.67	30.17 ± 4.76
Imidacloprid 3.67mg / kg / day	6.01 ± 1.77	24.33 ± 7.55
Imidaclopride 5.18 mg/kg/jour	6.22 ± 1.32	27.33 ± 6.55

However the microscopic observation of the histological sections of the thyroid carried out by **(Martoga and Martoga;1967)**, showed histopathology in the treated series compared to the control series**. (Figure 16):** A and B shows that the thyroid tissue of the control series composed of numerous follicles of variable diameter and each is delimited by a simple epithelium composed of thyreocytes with black and ovoid nuclei, These cells surround a colloid with large filled light. Treatment with imidacloprid affects the histological structure of the thyroid for the two doses tested. These alterations result in many degenerative changes of varying degrees in many regions of the thyroid **(Figure 14: C and E)**. By thereby increasing the number of micro follicles, the number of follicular and parafollicular cells leading to thyroid cancer. Colloid loss and presence of necrotic tissue are also recorded **(Figure 14: D and F)** based on histopathological examination and hormonal dosage results **(Saadi and *al.*, 2014**) suggest that the two doses tested imidacloprid caused an increase in an inflammatory process in the thyroid gland which was a sign of toxicity in rats.

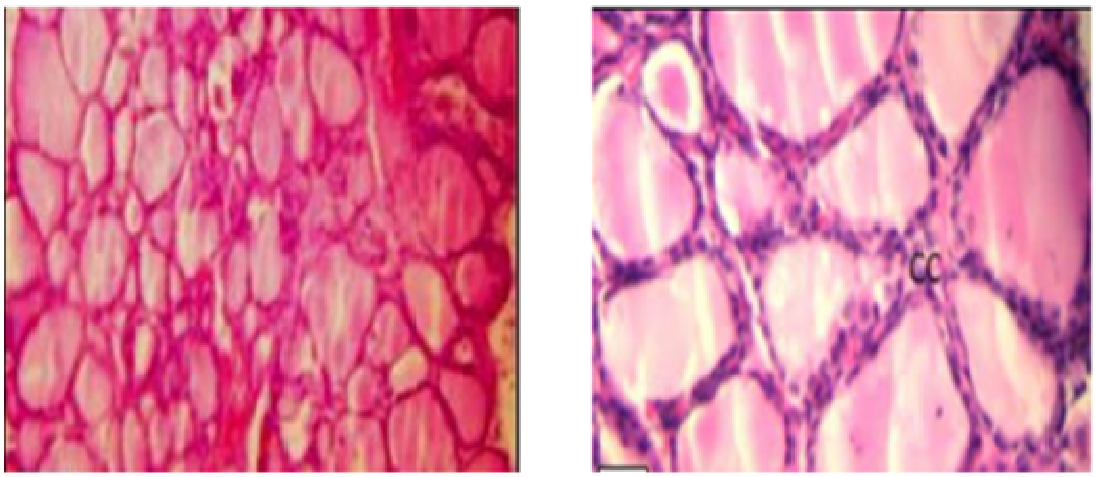


Figure 13: Histological section of a thyroid tissue from the control series, (A) 100 x, (B) 400x **(Saadi and *al.*,2014).**

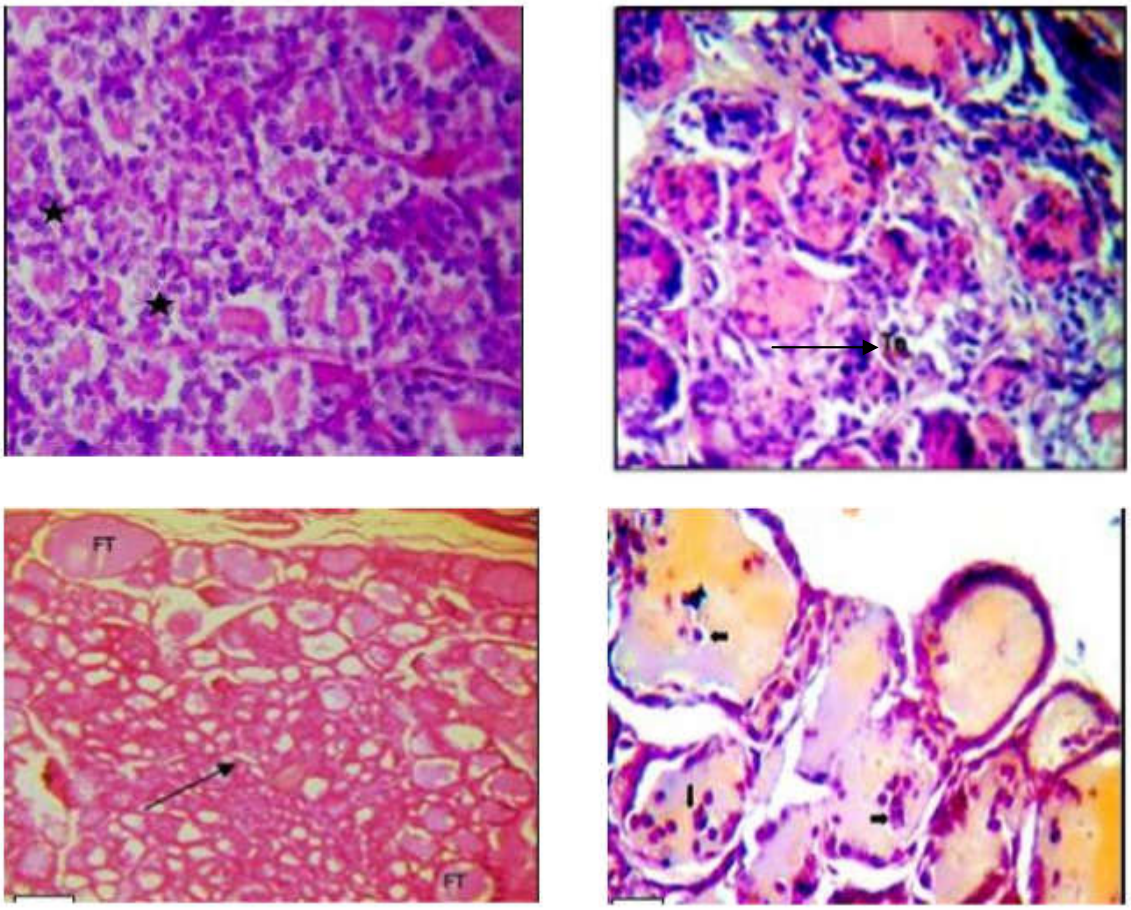


Figure 14: Histological section of a thyroid tissue, (C) 100x: series treated with imidacloprid at 3.67 mg / kg / day, (E) 100x: series treated with imidacloprid at 5.18 mg / kg / day in wistar rats, (D) and (F) image 400x showing the loss of colloid and the presence of necrotic tissue. **(Saadi and *al.*, 2014).**

CC: C cells. Tn: Necrotic Tissue; Indicates the loss of colloid and the increase in follicular cells. Indicates the presence of dead cells.

7.Non-carcinogenic effect:

7.1. The reproduction includes all

The stages which go from the production of gametes conditioning fertility, up to the sexual maturity of individuals through fertilization and implantation of the egg, then embryonic and fetal development. All these stages are characterized by numerous cell divisions extremely sensitive to environmental agents **(Multigner, 2005).** The effects of neonicotinoids on the reproductive process has been proven expensive in rabbits, indeed show the increase in testosterone in subjects exposed to neonicotinooid in an agricultural environment**. (Robitaille,2014**) Studies have shown the alteration of hormones involved in

the reproductive process but also follicular development in rabbits exposed to acetamiprid**. (Aïna and *al.*,2015)** The effect of neonicotinoids on the reproductive process in rats remains unclear. Experiments on the wistar rat under laboratory conditions have shown the impact of a neonicotinoid imidacloprid on reproduction. **(Nabiuni and *al.*,2015)** performed on the wistar rat under laboratory conditions have shown the impact of a neonicotinoid imidacloprid on reproduction. The study was continued for 20 days before the administration of imidacloprid by ingestion in food. In order to study the toxicity of this pesticide on the reproductive process, a blood sample was taken to measure the following hormones, testosterone and progesterone which were determined by the Immuno Chemiluminescence Test. So in order to determine follicular development, histological sections of the follicles were made**(Martoga andMartoga, 1967).** The follicles are classified according to the morphology and the diameter into 6 groups: primordial, primary, secondary, Graaf, and the corpus luteum (Nabiuni and al., 2015). The results of this experiment confirm a significant decrease in the level of testosterone and progesterone compared to the controls **(Table 03).** The treatment of rats with imidacloprid affects the number of ovarian follicles **(Figure 18)** but also the diameter of these follicles **(Figure 19)** compared to the control series, thereby altering the reproductive process.

Table 03: The effects of imidacloprid treatment on serum progesterone and testosteron in wistar rats compared to controls. **(Nabiuni and *al.*, 2015).**

Hormone	Control	Imidacloprid
Testosterone (ng/ml)	0,16	0.15
Progesterone (ng / ml)	7.22	5.03*

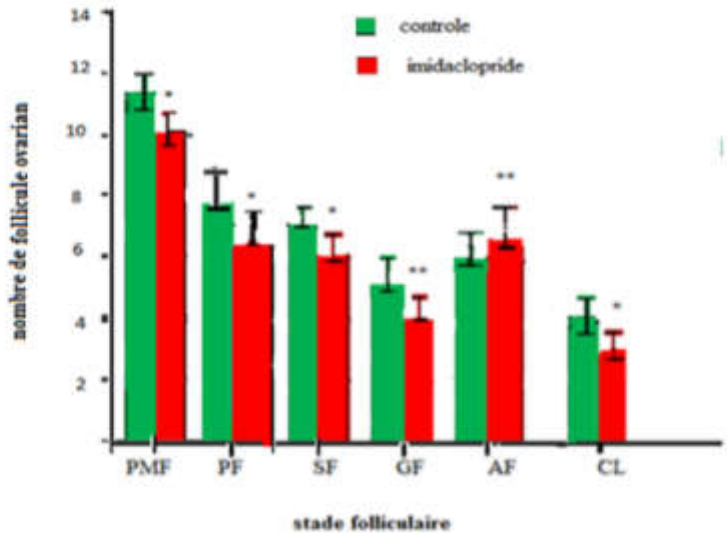
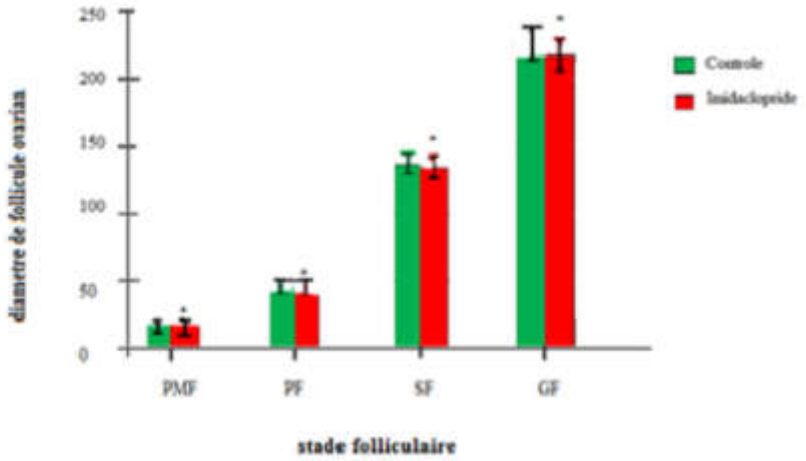


Figure 15: Effect of imidacloprid on follicular number in the wistar rat. PMF: primordial follicles, PF: primary follicle, SF: secondary follicle, GF: Graaf follicle, AF: atretic follicle, CL: corpus luteum



(Nabiun, and *al.,* 2015).

Figure 16: Effect of imidacloprid treatment on follicle diameter in wistar rats. PMF: primordial follicle, PF: primary follicle, SF: secondary follicle, GF: Graaf follicle, AF: atretic follicle, CL: corpus luteum **(Nabiuni, and *al.,* 2015).**

7.2.Hepatotoxic effect of neonicotinoids:

The immune system is a biological system made up of a coordinated sandof recognition and defense elements that discriminate between the self and the non-self. It is made up of a complex interaction system involving many different organs, cells and substances. The bone marrow and thymus produce immune cells, lymphocytes. many pesticides are known to have immunotoxic effects **(Riedel and *al.,* 1997).** New pesticides are therefore developed each year to better meandhealth requirements. and other, each year new pesticides are withdrawn

from the markandor put on the watch list due to their high toxicity on non-targandorganisms **(Yahia, 2016).** Studies have shown the effect of a neonicotinoid acetamiprid on the number of lymphocytes. **(Aina and *al.*,2015)**. showing the increase in lymphocytes in rabbits exposed to another compound belongs to the family of neonicotinoids, thiamethoxam. **(Moullan and *al.*,2016)** The hematological effects of neonicotinoids remains poorly understood, studies have shown the effects of a neonicotinoid acetamiprid in wistar rats. **(Mondal and *al.*, 2009)** This pesticide has been tested orally at three concentrations 25, 100 and 200 mg / kg / day for 28 days to assess chronic toxicity. After a blood sample from the control and treated series, two types of cells were studied: neutrophils and lymphocytes. The results obtained showed a significant increase ($p \leq 0.01$) in the total number of neutrophils and a significant decrease ($p \leq 0.01$) in the total number of lymphocytes compared to the control series and those with the two doses tested and a greater effect of the high dose 200 mg/kg/jour **(Table 04**).

Table 04: Effect of a neonicotinoid acetamiprid on the number of lymphocytes and neutrophils in the wistar rat **(Mondal and *al.*, 2009).**

Settings	Witnesses	25 mg/kg/jour	100 mg / kg / day	200 mg/kg/jour
Neutrophil (%)	20.95 ± 0.57	23.17± 0.70	26.21± 0.47	27.17± 0.67
Lymphocyt (%)	71.57± 0.57	67.17± 0.47	64.75± 0.65	63.0 ± 1.18

7.3.Effect of neonicotinoids on liver function:

The liver is an organ of the digestive system that performs three main functions storage, redistribution, synthesis and purification after the digestion / adsorption process **(Desvergne and *al.*, 2006).** The liver is also a detoxifying organ which biotransformed many xenobiotics with a view to their elimination **(Desvergne and *al.*, 2006).** The most significant exams showing inflammation of the liver are transaminases, Glutamopyruvate Transferase (TGO), Aspartate aminotransferase (ASAT), Glutamooxaloacetate Transferor (TGP) and alanine aminotransferase (ALAT). These are enzymes that are released into the blood during the destruction of liver cells (cytolysis) due to inflammation, for example, from a viral infection (viral hepatitis) or poisoning. But also conjugated bilirubin (direct), which marks the destruction of liver cells and free bilirubin (indirect), which can come from the destruction of red blood cells. Alkaline phosphatases, fibrinogen and albumin are parameters that indicate good or bad liver function. Research into the toxicity of a neonicotinoid imidcloprid has

reported alterations in the activity of liver enzymes and increased enzyme activity of alkaline phosphatase in rats. Results revealed significantly elevated levels of transaminases, (TGO) and (TGP) and bilirubin levels compared to controls, recorded an increase in bilirubin levels in rats treated with neonicotinoids oral acetamiprid at three concentrations 25, 100 and 200 mg / kg / day for 28 days compared to controls **(Table 05).(Bhardwaj and *al.,* 2010.,Mondal and *al.*,(2009)**

Table 05: Effect of a neonicotinoid acetamiprid on the bilirubin level in the wistar rat **(Mondal and *al.,* 2009).**

Setting	Witnesses	25mg/kg/day	100mg/kg/day	90mg/kg/day
Bilirubin	1,01±0,10	1,17±0,12	1,28± 0,08	1,75± 0,19

7.4. Effect of neonicotinoids on antioxidant activity:

The detoxification system involving detoxification enzymes remains the only means of studying the toxicity of xenobiotics. The enzymes involved are mainly divided into: phase I enzymes, functionalization site (oxidases, reductases, hydrolases) making it possible to mask the electrophilic or nucleophilic elements of the xenobiotic. Phase II enzymes, known as conjugation enzymes, which fix endogenous hydrophilic derivatives (glutathione, glucoside, phosphate or glycuronic acid) on the functional groups revealed in phase I, these conjugation enzymes are transferases, the best known of which are glutathione-S transferases (GSTs). Studies have shown that a detoxification system has been established in rats after the administration of a neonicotinoid imidaclipride orally at two doses 45 and 90 mg / kg. In fact, an increase in the activity of two detoxifying enzymes, GSH and GST, was recorded compared to the controls **(Table 06).**

Table 06: Effect of imidacloprid on the activity of glutathione (GSH) and glutathione S-transferase (GST), administered orally at 45 mg / kg and 90 mg / kg **(Lonare and *al.,* 2014).**

Settings (µmol / h / mg / protein)	GST	GSH
Control	0.45± 0.02	47.89± 1.24
Imidaclopride 45mg/kg	0.41 ±0.02	42.31± 1.39
Imidaclopride 90mg/kg	0.37± 0.03	38.72± 1.76

7.5. Neurotoxic effect of neonicotinoids:

The impact of neonicotinoids has been reported to be 20mg / kg / day of oral imidacloprid. Their results showed the inhibition of the specific activity of acetylcholinesterase (AChE). **(Bhardwaj and *al.,* 2010)** Similar results have been reported to show the alteration of this key nervous system enzyme after administration of the pendimethaline compound to rats. **(Rodrigues and *al.,* 2010) (Banerjee, and *al.,*2014)** successfully altered this key nervous system enzyme after administration of the pendimethalin compound to rats. However, the intraperitoneal injection of imidacloprid at a dose of 337 mg / kg / day for 30 days causes an increase in the specific activity of this enzyme in rats, thereby inducing alteration of the nervous system and muscular tetany. **(Abou-Donia and *al.,* 2008).** Recent work has reported a significant decrease in acetylcholinesterase in the plasma and brain of rats treated orally with imidacloprid compared to the control series **(Table 07).**

Table 07: Effect of imidacloprid on the activity of acetylcholinesterase in the plasma and brain of rats, administered orally at 45 mg / kg and 90 mg / kg **(Lonare and *al.,* 2014).**

Treatment	Plasma	Brain
Control	2.52 ±0.09	11.96 ± 0.55
Imidacloprid 45mg / kg	2.37± 0.08	9.77 ± 0.19
Imidaclopride 90mg/kg	0.39 ±0.01	7.68 ± 0.16

Chapter 03 :

Imidacloprid

1. Definition:

Imidacloprid is a neurotoxic insecticide, which belongs to the group of neonicotinoids. . It is approved as a plant protection product to fight against harmful insects on agricultural crops and nurseries, garden plants and houseplants and also, as an external pest control on pets, . It is also referenced as a biocidal product. **(Meister, 1995).**

2. Chemical and class type:

- Imidacloprid is a neonicotinoid insecticid in the chloronicotinyl nitroguanidine chemical family. **(Tomlin,.2006) (Tomlin,.2006)** The International Union of Pure and Applied Chemistry (IUPAC) name is 1-(6-chloro-3- pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine and the Chemical Abstracts Service (CAS) registry number is 138261-41-3.**(Tomlin,.2006)**
- Neonicotinoid insecticide are synthetic derivatives of nicotine, an alkaloid compound found in the leaves of many plants in addition to tobacco**.(Costa.,2008) (Copping.,2001) (Ware; Whitacre, 2004)**
- Imidacloprid was first registered for use in the U.S. by the United States Environmental Protection
- Agency (U.S. EPA) in 1994 **(Figure20) . (Hovda, L. R.; Hooser, S. B,.2002)**

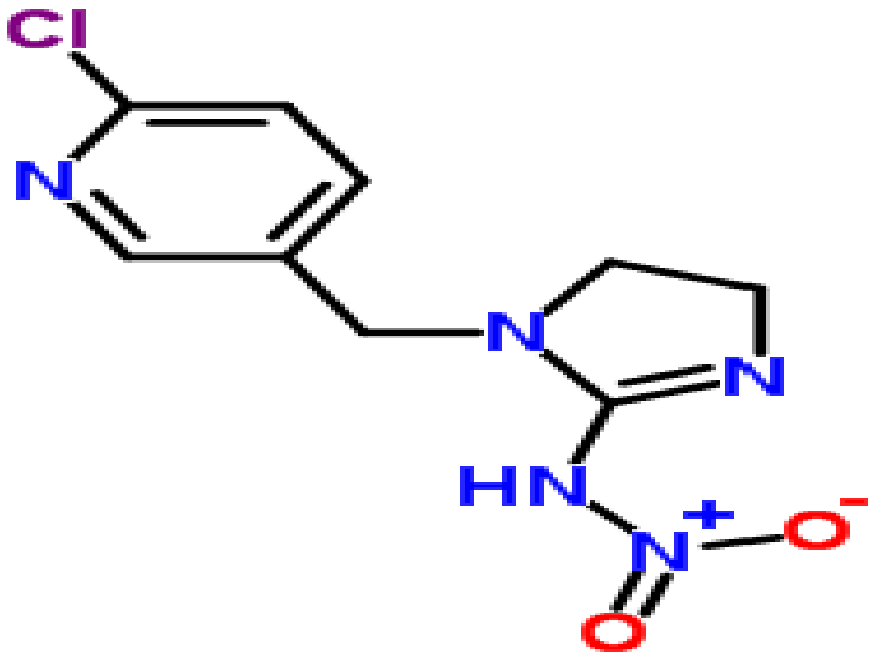


Figure17: Molecular Structure - Imidacloprid (https://www.chemsrc.com)

3.Physical and Chemical properties:

Imidacloprid is made up of colorless crystals with a slight but characteristic odor. **(Tomlin, 2006)**

Octanol-Water Partition Coefficient (K_{ow}) : 0.57 at 21 °C

Henry's constant :1.7 x 10^{-10} Pa·m^3/mol

Molecular formule: C9H10ClN5O2

Molecular weight: 255.7

CAS #: 13826-41-3

IUPAC name: 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine.

C.A. name: 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine.

Form: Colorless crystals with a weak characteristic odor.

Melting point: 143.8 degrees C (crystal form 1) 136.4 degrees C (crystal form 2).

Solubility in water: 0.51 g/l (20 degrees C).

Solubility in other solvents @ 20 degrees C: dichloromethane - 50.0 - 100.0 g/l; isopropanol - 1.0-2.0 g/l; toluene - 0.5-1.0 g/l; n-hexane - <0.1 g/l; fat - 0.061 g/100g.

Vapor pressure: 0.2 uPa (20 degrees C) (1.5 X 10 to the minus 9 mmHg**).**

Specific gravity/density: 1.543 (20 degrees C).

Stability: Stable to hydrolysis at pH 5-11.

Melting point: 136.4-143.8 degrees C.

Kow log p: 0.57 (22 degrees C). (U.S. Environmental Protection Agency,. 1995)

Soil Sorption Coefficient (K_{oc}) **(Oliver and *al*.,2005)**: 156-960, mean values 249-336.

4. Mode of Action:

4.1. TargandOrganisms:

- Imidacloprid is designed to be effective by contact or ingestion. **(Tomlin, ,.2006)**It is a systemic insecticide that translocates rapidly through plant tissues following application. **(Tomlin, ,.2006) (Fossen,2006)**

- Imidacloprid acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system.**(Buckingham and *al.,* 1997) (Matsuda, and *al.,*2005).** In insects, these receptors are located only within the central nervous system. Following binding to the nicotinic receptor, nerve impulses are spontaneously discharged at first, followed by failure of the neuron to propagate any signal**. (Schroeder and *al.,* 1984) (Sheets,2001)** Sustained activation of the receptor results from the inability of acetylcholinesterases to break down the pesticide**. (Sattelle and *al.,* 2005)**. This binding process is irreversible. **(Ware and *al.,* 2004)**

4.2. Non-targandOrganisms:

- Imidacloprid's mode of action is similar on targandand non-targandbeneficial insects including honeybees, predatory ground beetles and parasitoid wasps. **(Fossen, ,2006)** However, imidacloprid is ineffective against spider mites and nematodes. **(Tomlin,2006)**
- Mammalian nicotinic receptors are made up of a number of subtypes. **(Sheets,2001)** In contrast to insects, these receptors are present at neuromuscular junctions as well as in the central nervous system. **(Sheets,2001)** However, the binding affinity of imidacloprid at the nicotinic receptors in mammals is much less than that of insect nicotinic receptors. **(Tomizawa and *al.,*1999)** This appears to be true of other vertebrate groups including birds**. (Matsuda and and *al.,* 1998)(Casida, and *al.,* 2005)**
- The blood-brain barrier in vertebrates blocks access of imidacloprid to the central nervous system, reducing its toxicity. **(Sheets,2001)**

5. Toxicological effect:

5.1. Acute toxicity:

Imidacloprid is moderately toxic. The oral dose of technical grade imidacloprid that resulted in mortality to half of the test animals (LD50) is 450 mg/kg body weight in rats **(Meister,1995),** and 131 mg/kg in mice **(U.S. Environmental Protection Agency,. 1995)**. The 24-hour dermal LD50 in rats is >5,000 mg/kg. It is considered non-irritating to eyes and skin (rabbits), and non-sensitizing to skin (guinea pigs) **(U.S. Environmental Protection Agency,. 1995)**. Some granular formulations may contain clays as inert ingredients that may act as eye irritants. In acute inhalation toxicity tests with rats, the airborne concentration of imidacloprid that resulted in mortality to half of the test organisms (LC50) is > 69 mg/meters cubed air in the form of an aerosol, and >5323 mg/meters cubed air in the form of dust. These

values represent the maximum attainable airborne concentrations **(U.S. Environmental Protection Agency,. 1995)**.

5.1.1. Signs of Toxicity – Animals:

- Salivation and vomiting have been reported following oral exposure. **(Tomlin,.2006) (Hovda.; Hooser,.2002)** Very high oral exposures may lead to lethargy, vomiting, diarrhea, salivation, muscle weakness and ataxia, which are all indicative of imidacloprid's action on nicotinic receptors. **(Tomlin,.2006)** Other signs of exposure at high doses are uncoordinated gait, tremors, and reduced activity. **(Thyssen; Machemer,1999)**
- Hypersensitivity reactions in skin have been reported following dermal applications of products containing imidacloprid. **(Tomlin,.2006)**
- Onsandof signs of toxicity is rapid following acute exposure. In rats, clinical signs of intoxication occurred within 15 minutes of oral exposure. **(Sheets,,2001) (Sheets,2002)** Signs of toxicity disappear rapidly, with most resolving within 24 hours of the exposure. Lacrimation and urine staining may persist for up to four days after exposure to some neonicotinoids. Death occurred within 24 hours following administration of lethal doses.
- Neither persistent neurotoxic effects nor effects with a delayed onsandhave been reported for imidacloprid. **(Sheets,2002)**

5.1.2. Signs of Toxicity – Humans:

- Three case reports of attempted suicides described signs of toxicity including drowsiness, dizziness, vomiting, disorientation, and fever. **(Wu; Lin; and *al.*,2001) (Shadnia,2007) (Deepu and *al.*, 2007)** In two of these cases, the authors concluded that the other ingredients in the formulated product ingested by the victims were more likely to account for many of the observed signs**. (Shadnia,2007)**
- A 69-year-old woman ingested a formulated product containing 9.6% imidacloprid in N-methyl pyrrolide solution. The woman suffered severe cardiac toxicity and death 12 hours after the exposure**. (Huang and *al.*, 2006)** Signs of toxicity soon after the ingestion included disorientation, sweating, vomiting, and increased heart and respiratory rates.**(Huang and *al.*, 2006)**
- A 24-year-old man who accidentally inhaled a pesticide containing 17.8% imidacloprid while working on his farm was disoriented, agitated, incoherent, sweating and breathless following the exposure. **(Agarwal, 2008)**

- Pandowners have reported contact dermatitis following the use of veterinary products containing imidacloprid on their pets.

5.2.Chronic Toxicity:

A 2-year feeding study in rats fed up to 1,800 ppm resulted in a No Observable Effect Level (NOEL) of 100 ppm (5.7 mg/kg body weight in males and 7.6 mg/kg in females). Adverse effects included decreased body weight gain in females at 300 ppm, and increased thyroid lesions in males at 300 ppm and females at 900 ppm. A 1-year feeding study in dogs fed up to 2,500 ppm resulted in a NOEL of 1,250 ppm (41 mg/kg). Adverse effects included increased cholesterol levels in the blood, and some stress to the liver (measured by elevated liver cytochrome p-450 levels) **(Federal Register,1995).**A three generation reproduction study in rats fed up to 700 ppm imidacloprid resulted in a NOEL of 100 ppm (equivalent to 8 mg/kg/day) based on decreased pup body weight observed at the 250 ppm dose level **(Federal Register,1995).**

5.2.2 Teratogenic Effects:

A developmental toxicity study in rats given doses up to 100 mg/kg/day by gavage on days 6 to 16 of gestation resulted in a NOEL of 30 mg/kg/day (based on skeletal abnormalities observed at the next highest dose tested of 100 mg/kg/day) (Pike, K.S and al.,1993). In a developmental toxicity study with rabbits given doses of imidacloprid by gavage during days 6 through 19 of gestation, resulted in a NOEL of 24 mg/kg/day based on decreased body weight and skeletal abnormalities observed at 72 mg/kg/day (highest dose tested) **(Federal Register,1995).**

5.2.3. Mutagenic Effects:

Imidacloprid may be weakly mutagenic. In a battery of 23 laboratory mutagenicity assays, imidacloprid tested negative for mutagenic effects in all but two of the assays. It did test positive for causing changes in chromosomes in human lymphocytes, as well as testing positive for genotoxicity in Chinese hamster ovary cells **(Federal Register, 1995).**

5.2.4.Carcinogenic Effects:

Imidacloprid is considered to be of minimal carcinogenic risk, and is thus categorized by EPA as a "Group E" carcinogen (evidence of noncarcinogenicity for humans). There were no carcinogenic effects in a 2-year carcinogenicity study in rats fed up to 1,800 ppm imidacloprid **(U.S. Environmental Protection Agency. 1995).**

5.2.5. Organ toxicity:

In short-term feeding studies in rats, there were thyroid lesions associated with very high doses of imidacloprid **(Federal Register, 1995).**

5.2.6. Fate in humans and animals:

5.2.6.1. Animals:

- Rats consumed imidacloprid in their diandfor three months at doses of 14, 61, and 300 mg/kg/day for males and 20, 83, and 420 mg/ kg/day for females. Researchers noted reductions in body weight gain, liver damage, and reduced blood clotting function and platelandcounts at 61 mg/kg/day in males and 420 mg/kg/day in females. Liver damage disappeared after exposure ended, but abnormalities in the blood were not entirely reversible. Researchers estimated the NOAEL at 14 mg/kg/day. **(Eiben and *al*.,1989)**
- Imidacloprid dust was administered through the noses of rats for six hours a day, five days a week for four weeks at concentrations of 5.5, 30.0, and 190.0 mg/m^3. Male rats exhibited reduced body weight gain at the two highest doses and at the highest dose, increased liver enzyme activity and increased blood coagulation time was noted. Female rats exhibited increased liver enzyme activity at the two highest doses and at the highest dose, researchers noted enlarged livers and reduced thrombocyte counts. No effects were observed at the lowest dose. **(Pauluhn,1988)**
- Researchers applied a paste containing 1000 mg/kg imidacloprid to the shaved flanks and backs of rabbits, exposing the rabbits for 6 hours a day for 15 days. Rabbits showed no effects from the treatment. **(Flucke,1990)**
- Researchers fed imidacloprid to beagles for one year. Concentrations were 200, 500, or 1250 ppm for the first 16 weeks and 200, 500, and 2500 ppm for the remainder of the trial. Doses were equivalent to 6.1, 15.0, and 41.0 or 72.0 mg/kg/day. Researchers noted reduced food intake in the highest dose group. Females in this group exhibited increased plasma cholesterol concentrations at 13 and 26 weeks. Both males and females in this group exhibited increased cytochrome P450 activity in the liver and

increases in liver weights at the end of the study. No adverse effects were observed at the two lowest doses. **(Allen;1989,1990)**

5.2.6.2. Huamns:

- No studies were found involving human subjects chronically exposed to imidacloprid**.**

6. Carcinogenicity:

6.1. Animals:

- Researchers concluded that Scottish terriers treated with topical flea and tick products, including those containing imidacloprid, did not have a greater risk of developing urinary bladder cancer compared with control dogs. **(Raghavan, 2004)** Rats were fed imidacloprid for 18 or 24 months at unspecified concentrations. Although signs of toxicity were noted, researchers concluded that imidacloprid showed no evidence of carcinogenic potential. **(Thyssen, 1999)**
- A range of studies using both *in vitro* and *in vivo* techniques concluded that imidacloprid did not damage DNA.

6.2. Humans :

- The U.S. EPA has classified imidacloprid into Group E, no evidence of carcinogenicity, based on studies with rats and mice. **(Thyssen, 1999) (*Federal Register*2005)**
- A study of human lymphocytes exposed to greater than 5200 μg/mL of imidacloprid demonstrated a slight increase in chromosome abnormalities *in vitro*, but this result wasnot found with *in vivo* tests.

7. Ecological effect:

7.1. Effect on birds:

Imidacloprid is toxic to upland game birds. The LD50 is 152 mg/kg for bobwhite quail, and 31 mg/kg in Japanese quail **(Meister, 1995) (U.S. Environmental Protection Agency,. 1995).** In studies with red- winged blackbirds and brown-headed cowbirds, it was observed that birds learned to avoid imidacloprid treated seeds after experiencing transitory gastrointestinal distress (retching) and ataxia (loss of coordination). It was concluded that the risk of dietary exposure to birds via treated seeds was minimal. Based on these studies, imidacloprid appears to have potential as a bird repellent seed treatment **(Avery, and *al*., 1994) (Averyand *al*.,1993).**

7.2. Effect on aquatic organism:

The toxicity of imidacloprid to fish is moderately low. The 96-hour LC50 of imidacloprid is 211 mg/l for rainbow trout, 280 mg/l for carp, and 237 mg/l for golden orfe. In tests with the aquatic invertebrate Daphnia, the 48- hour EC50 (effective concentration to cause toxicity in 50% of the test organisms) was 85 mg/l. **(U.S. Environmental Protection Agency,. 1995)** .Products containing imidacloprid may be very toxic to aquatic invertebrates.

7.3. Effects on other animals (Non targandspecies):

Imidacloprid is highly toxic to bees if used as a foliar application, especially during flowering, but is not considered a hazard to bees when used as a seed treatment **(U.S. Environmental Protection Agency,. 1995).**

8. Enironmental fate:

8.1. Breakdown of chemical in soil and groundwater:

The half-life of imidacloprid in soil is 48-190 days, depending on the amount of ground cover (it breaks down faster in soils with plant ground cover than in fallow soils) **(Scholz and *al*., 1992).** Organic material aging may also affect the breakdown rate of imidacloprid. Plots treated with cow manure and allowed to age before sowing showed longer persistence of imidacloprid in soils than in plots where the manure was more recently applied, and not allowed to age **(Rouchard and *al.,* 1994).** Imidacloprid is degraded stepwise to the primary metabolite 6-chloronicotinic acid, which eventually breaks down into carbon dioxide **(Buckingham and *al*.,1997).** There is generally not a high risk of groundwater contamination with imidacloprid if used as directed. The chemical is moderately soluble, and has moderate binding affinity to organic materials in soils. However, there is a potential for the compound to move through sensitive soil types including porous, gravelly, or cobbly soils, depending on irrigation practices **(Matsuda and *al*,.2005).**

8.2. Breakdown of chemical in surface water:

- Imidacloprid is broken down in water by photolysis. **(Liu and *al*., 2006)** Imidacloprid is stable to hydrolysis in acidic or neutral conditions, but hydrolysis increases with increasing alkaline pH and temperature. **(Zheng and *al.,* 1996)**
- Researchers determined that hydrolysis of imidacloprid produced the metabolite 1-[(6-chloro-3-pridinyl)methyl]-2-imidazolidone. **(Zheng and *al.*,1996)** This may be further broken down via oxidative cleavage of the N-C bond between the pyridine and

imidazolidine rings, and the resulting compounds may be broken down into $C0_2$, $N0_3^-$, and Cl^-.**(Liu and *al.,* 2006)**

- When imidacloprid was added to water at pH 7 and irradiated with a xenon lamp, half of the imidacloprid was photolyzed within 57 minutes. **(Roberts and *al.,* 1999)** Nine metabolites were identified in the water, of which five were most prominent. These included a cyclic guanidine derivative, a cyclic urea, an olefinic cyclic guanidine, and two fused ring products. These metabolites accounted for 48% of the radio carbon label following two hours of radiation, and the parent compound accounted for 23% of the label. **(Roberts and *al.,* 1999)**
- Although hydrolysis and photodegradation proceeded along different metabolic pathways in aqueous solution, the main metabolite was imidacloprid-urea in both cases. **(Liu and *al.*,2006)**
- At pH 7, only 1.5% of the initial concentration of 20 mg/L of imidacloprid was lost due to hydrolysis in three months, whereas at pH 9, 20% had been hydrolyzed in samples that were kept in darkness for the same time period. **(Zheng and *al.*,1996)**
- The presence of dissolved organic carbon in calcareous soil may decrease the sorption potential of imidacloprid to soil, and thus increase the potential for imidacloprid to leach and contaminate groundwater. **(Flores-Cespedes and *al.,* 2002)**
- A total of 28.7% of imidacloprid applied to a 25 cm soil column in the laboratory was recovered in leachate. Formulated products showed greater rates of leaching likely due to the effects of carriers and surfactants. Under natural conditions, soil compaction and rainfall amount may also affect leaching potential. **(Gupta and *al.,* 2002)**
- Imidacloprid is not expected to volatilize from water.**(*(HSDB), Imidacloprid*,2006)**

8.3. Breackdown of chemical in vegetation:

Imidacloprid penetrates the plant, and moves from the stem to the tips of the plant. It has been tested in a variety of application and crop types, and is metabolized following the same pathways. The most important steps were loss of the nitro group, hydroxylation at the imidazolidine ring, hydrolysis to 6- chloronicotinic acid and formation of conjugates **(U.S. Environmental Protection Agency,. 1995)**

9. Fate in the body:

9.1. Absorption:

- The gastrointestinal tract of rats absorbed 92% of an unspecified dose. Plasma concentrations peaked 2.5 hours after administration.
- Little systemic absorption through the skin occurs following dermal exposure in pets.
- Researchers tested imidacloprid absorption using human intestinal cells. Cells rapidly absorbed imidacloprid at a very high rate of efficiency. Researchers concluded that an active transport system was involved. **(Brunandand *al.,* 2004)**

9.2. Distribution:

- Researchers administered a single oral dose of radio-labeled imidacloprid at 20 mg/kg to male rats. One hour after dosing, imidacloprid was detected throughout the bodies with the exception of fatty tissues and the central nervous system**. (Klein and *al*., 1987)**
- No studies were found examining the distribution of imidacloprid in humans.

9.3. Metabolism:

- Mammals metabolize imidacloprid in two major pathways discussed below. Metabolism occurs primarily in the liver. **(Thyssen and *al* 1999)**
- In the first pathway, imidacloprid may be broken by oxidative cleavage to 6-chloronicotinic acid and imidazolidine. Imidazolidine is excreted in the urine, and 6-chloronicotinic acid undergoes further metabolism via glutathione conjugation to form mercaptonicotinic acid and a hippuric acid**. (Thyssen and *al*.,1999) (Klein and *al*., 1990)**
- Imidacloprid may also be metabolized by hydroxylation of the imidazolidine ring in the second major pathway. **(Thyssen and *al*., 1999) (Klein and *al*., 1990)** Metabolic products from the second pathway include 5-hydroxy and olefin derivatives. **(Klein,1987)**

9.4. Excretion :

- The metabolic products 5-hydroxy and olefin derivatives resulting from hydroxylation of the imidazolidine ring are excreted in both the feces and urine. **(Klein and *al*., 1990) (Schulz-Jander and *al*., 2002)**

- Metabolites found in urine include 6-chloronicotinic acid and its glycine conjugate, and accounted for roughly 20% of the original radio-labeled dose. **(Roberts and *al*., 1999)**
- Metabolites in the feces accounted for roughly 80% of the administered dose in rats and included monohydroxylated derivatives in addition to unmetabolized imidacloprid, which made up roughly 15% of the total. Olefin, guanidine, and the glycine conjugate of methylthionicotinic acid were identified as minor metabolites**. (U.S. Environmental Protection Agency. 1995) (Roberts and *al*., 1999)**
- Rats excreted 96% of radio-labeled imidacloprid within 48 hours following an unspecified oral dosing, with 90% excreted in the first 24 hours. (Klein, O.1987) Radio-labeled imidacloprid was present in low amounts in organs and tissues 24 hours after male rats were orally dosed with 20 mg/kg. **(U.S.Environmental Protection Agency, 1995)**
- No information was found on the specific metabolism of imidacloprid in humans.

Chapter 04 :

Melissa Sp

1. Definition:

Mellissa has long been known for its effectiveness in depressive states and anxiety. It is part of the composition of "Carmes lemon balm water**"[Andrew Chevallier., 2007**] (made since the 17th century is an alcohol distilled from various plants, the main base of which is Mellissa officinalis) **(Gerard ,2003)** . This remedy, now developed by a pharmaceutical laboratory, according to the original recipe, is recommended in case of nervous disorders. Today, this fragrant and calming plant can be effective in the treatment and herpes. (**Andrew Chevallier.,2007) (figure 18)**

Figure 18 : *Mellissa officinalis* [https://www.homegrownherbalist.net]

2. Morphological description:

Lemon balm is a herbaceous perennial 30-80cm, hairless or hairy, has a penetrating and very pleasant lemon scent. **(Gerard., 2003)**

The underground part consists of underground stems, branching, bearing roots and producing adventitious buds which allow the plant to perpetuate and multiply. **(Hammoudi.,2015; Zeghib., 2013)**

The stem: It is erect, quadrangular (typical character of Lamiaceae), more or less hairy; little branched at the base, it becomes strongly in the upper parts at flowering.

The branches of the upper part bear flowers and are well developed, while they are short and non-flowering in the lower part. **(figure 19)(Hammoudi., 2015; Zeghib., 2013)**

Figure 19: The stem of mellissa *officinalis* **(https://www.sciencephoto.com)**

The leaf : The leaves of Melissa *officinalis* are simple, opposite, oval, sometimes slightly cordate, petiolate, broadly toothed in saw, with reticulated venation and measuring 5 to 8 cm y 4 to 5 cm. The upper surface, dark bright green in color, is rough to the touch because it is covered with fine, short, white protective hairs. The ribs, protruding on the underside much paler and hairless, form a network between the branches from which the blade is raised which gives the underside a characteristic embossed appearance. The leaves of the axillary twigs are smaller. **(Figure 20)(Hammoudi and *al*.,2015 ; Zeghib and *al*.,2013)**

Figure 20: The leaf of mellissa *officinalis* **(https://www.kaaterskillherbexchange.com)**

The flower : Flowering takes place from June to September. The type of inflorescence is the cyme; white, pink, briefly stalked. Les fleurs sont groupees par trois ou six en verticilles axillaires unilateraux, espaces le long de la tige andinseres à l'aisselle des feuilles superieures andcentrales**.(Figure 21)(Hammoudi R.,2015 , Zeghib A.,2013)**

Figure21: the flower of mellissa *officinalis* **(https://www.mesarbustes.fr)**

3. Botanical classification:

The botanical classification places Melissa officinalis in:

- Reign: Plantae
- Sub-Kingdom: Tracheobionta
- Division: Spermatophyta
- Phylum: Spermaphytes
- Under phylum: Angiosperms
- Class: True broadleaf
- Subclass: Asterids
- Order: Lamial
- Family: Lamiaceae
- kind: Melissa
- Species: Melissa *officinalis* **(Dupont F., Guignard J.L., (2007))**

The species Melissa *officinalis* known by the French vernacular name „Melisse, Citronnelle", Arabic „Tariane" and Marocain „hbak tranj"حبق طرنج

4. Main constituents:

The fresh lemon balm plant is composed of 0.01% essential oil, while the dry plant contains 0.05%. **(Youla and Latrous.,2017)**

The essential oil itself consists of:**((Youla and Latrous.,2017)**

- Terpene aldehydes (citronellal and citral, a mixture of neral and geranial)
-Alcohols, terpenics (eugenol, geraniol, citronellol, linalol)
- Sesquiterpenes (caryophyllene)

The other components of lemon balm are:

- Phenol acids (rosmarinic acid, chlorogenic acid, caffeic acid)
- Triterpenes (ursolic acid, oleanic acid, hydroxyoleanolic acid)
- Flavonoids (derivatives of luteolin and quercetol)
- Coumarines
- Tannins
- Uronic mucilages

5. Medicinal and therapeutic uses of *Melissa Officinalis*:

Lemon balm is a plant native to the east of the Mediterranean basin (Turkey) and found in all temperate climates on the planet. Its use, as a medicinal plant, dates back to Theophrastus and Hippocrates, in ancient Greece. At the time, we already recognized its benefits to calm anxious people and soothe nervous disorders. Arabs used it as an antispasmodic and Europeans as a digestive, calming and for antiviral treatment. **(Bartels.,1986 ; Quezel and *al.*,1962/1963)**

5.1. Internal uses:

-Treatment of nervous disorders: stress, anxiety, anxiety, nervous breakdown.
- Antispasmodic effects: spasms of the stomach and colon.
- Sleep disturbances: insomnia.
- Heart problems: tachycardia.
-Gastric disorders: excess stomach acidity.
- Improves blood circulation: distension or contraction of the vessels.

5.2. External uses:

-Fight against viral infections: cold and genital herpes, shingles, neuralgia and injuries.

- Relaxation of muscles and nerves: tense muscles and nerves.

5.3. Contraindications:

Due the lack of substantial documentation on the issue taking melissa is not recommendes for children as well as pregnant or breastfeeding women . taking melissa can accentuate the effects of alcohol on the recommended to consume alcohol during treatement with lemon balm (melissa) products

Second part

Practical side

Chapter 01:

Materials and methods

1. Materials and methods

1.1. Laboratory equipment:

The experimental research work was did at the laboratory in the University of Exact Sciences and Sciences of Nature and Life El Arbi Tebessi from the adaptation period, treatment, sacrifice and dosage.

1.2. Biological material

Choice of animal:

For our work we chose the wistar rats (are from the Pastor Institut of Algiers)

In the event that human physiology is targeted because they are sufficiently close mammals to humans.In addition, they are very soft ,small and it facilitates handling

Figure22: wistar rats **(original)**

1.3 Chemical material

We used the neonicotinoid pesticide of the substance imidaclopride

We practiced in two different doses, which the doses are:

- 1,45mg /kg ⟶ for 5mg of solution.
- 14,5mg /kg ⟶ for 50mg of solution.

1.4. Plant materiel :

During treatment, we used the aqueous extract of Melissa officinalis dilute in distilled water to facilitate oral gavage it is available in the laboratories of our university the dose eligible is 10mg /kg

Figure 23 : melissa *officinalis* extract (**provencearome.fr**)

2. Method

2 .1 Allotment and adaptation of rats

We used 20 rats globally for this study, they are taken to veterinary cages made of polyethylene woven sawdust in a pandstore favorable for them the rat food was: corn, bread and water

Figure24: housing of rats in cages during the experimental study **(Original)**

2.2. Weight measurement and processing:

The weight of the rats was measured daily by an electronic balance the results are in grams about 150 to 200 g; for each rat we distinguish a very precise dose of pesticides and Melissa Extract according to the following relationship.

290g ⟶ 150ul
Measured weight ⟶ dose obtained

2.3Treatment:

Our study is subacute lasted 20 days, the treatment is by oral voice is done by the order of the batches.

Lot Ct	Lot A	Lot B	Lot C	Lot D
4rat undergoes no treatment	4 rats are treated with pesticide D :50mg	4 rats are treated with pesticide D :5mg +Melissa	4 rats are treated with pesticide D :50mg+ Melissa	4 rats are treated with pesticide D :5mg

D: dose of pesticide neonicotinoide , Ct: control

Figure 25: weight measurement with a gram scale(Original)

Figure26: oral gavage of Melissa extract (original)

2.4. Behavioral assessment test

2.4.1.Labyrinthe classic:

This type of labyrinth consists of a platform with a series of vertical walls and a transparent ceilingThe rat starts in one place, runs through the labyrinth, to arrive at a food reward in another place in a given time**. (Figure 27)**

Figure27: classic labyrinth fixed by camera connected to a computer in a quiandroom. (Original)

2.4.2. Handling mode

We followed by a camera connected to a computer the movement of the rats to arrive at the food reward that varied from lot to other during the examination , To assess the animals' level of anxiety, we measure the time spent in the different parts of the device , thus, the principle of the test based on the fact that an animal explores the parts or places of initial deposit for longer, is considered to be anxious, the duration of the test is 20 min , and between each test the labyrinth is cleaned with lethanol 10%(**ku lli jaako-movits and al.,2005**)

2.4.3. The Vsoc-maze:

The behavioral studies using the vsoc labyrinth which we did to know the neurobiological mechanisms appeared in rats and evaluating the neuropsychiatric disorders to reach the effect of treatment in social relationships and reactions

Manual work

the Crawley's sociability test also called three-chamber apparatus **(Chadman and *al*., 2008;McFarlane and *al*., 2008; Moy and *al*., 2008)**, which allows to study two different parameters:sociability and preference for social novelty. Sociability is defined as the tendency of theexperimental mouse to spend more time exploring an unfamiliar mouse than exploring anobject or an empty chamber. Instead, preference for social novelty is defined as the propensityto spend more time exploring a new unfamiliar mouse than the now-familiar mouse that wasexplored in the sociability phase **(Moy and *al*., 2004; Yang and *al.,* 2011)**. Due to the length ofthe different phases in the Crawley's sociability test, the procedure is time-consuming andexperimental variability is sometimes troublesome.**(Martínez-Torres, and *al.,*2019)**

Figure 28: The Vsoc-maze fixed by camera connected to a computer in a quiandroom. (**Original)**

2.5. Sacrifice and organ harvesting:

After 20 days of treatment we made the sacrifice and the removal of the organs, we dissected the rat skull to extract the brain**. (Figure 29) (Figure 30)**

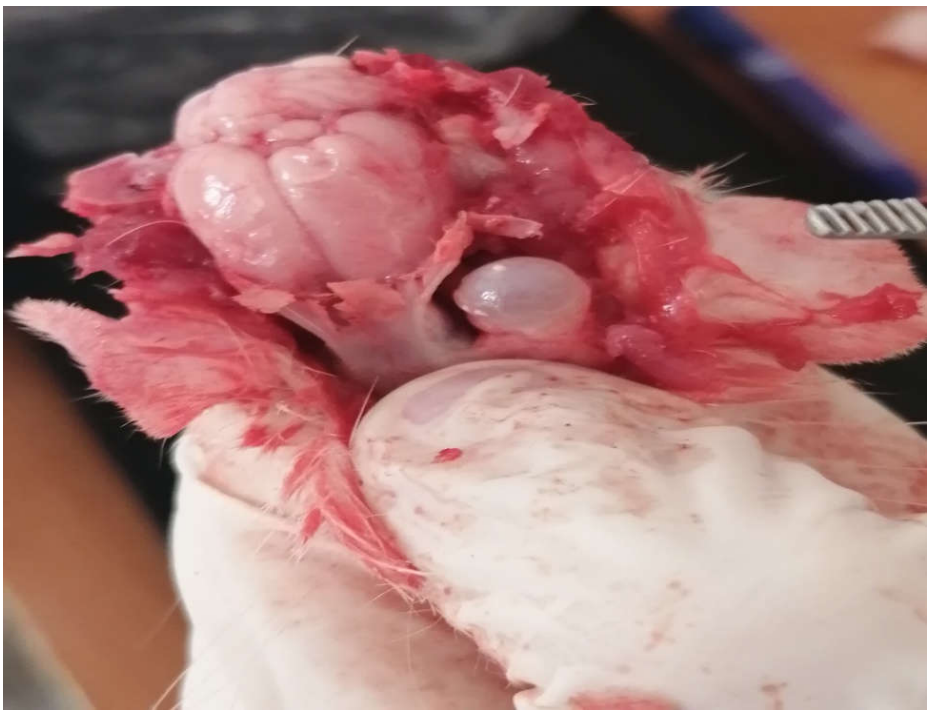


Figure 29: Removal of brain (Original)

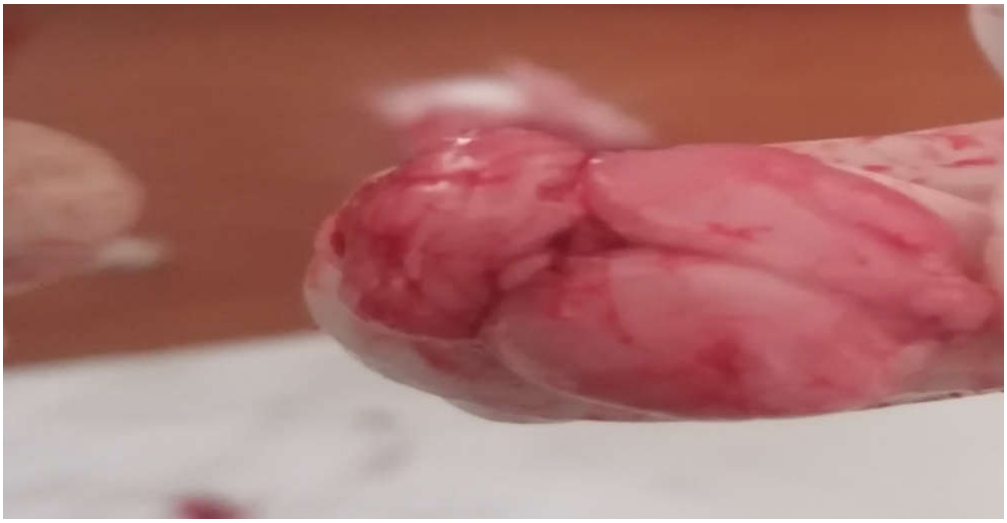


Figure 30:Rat brain sample (original)

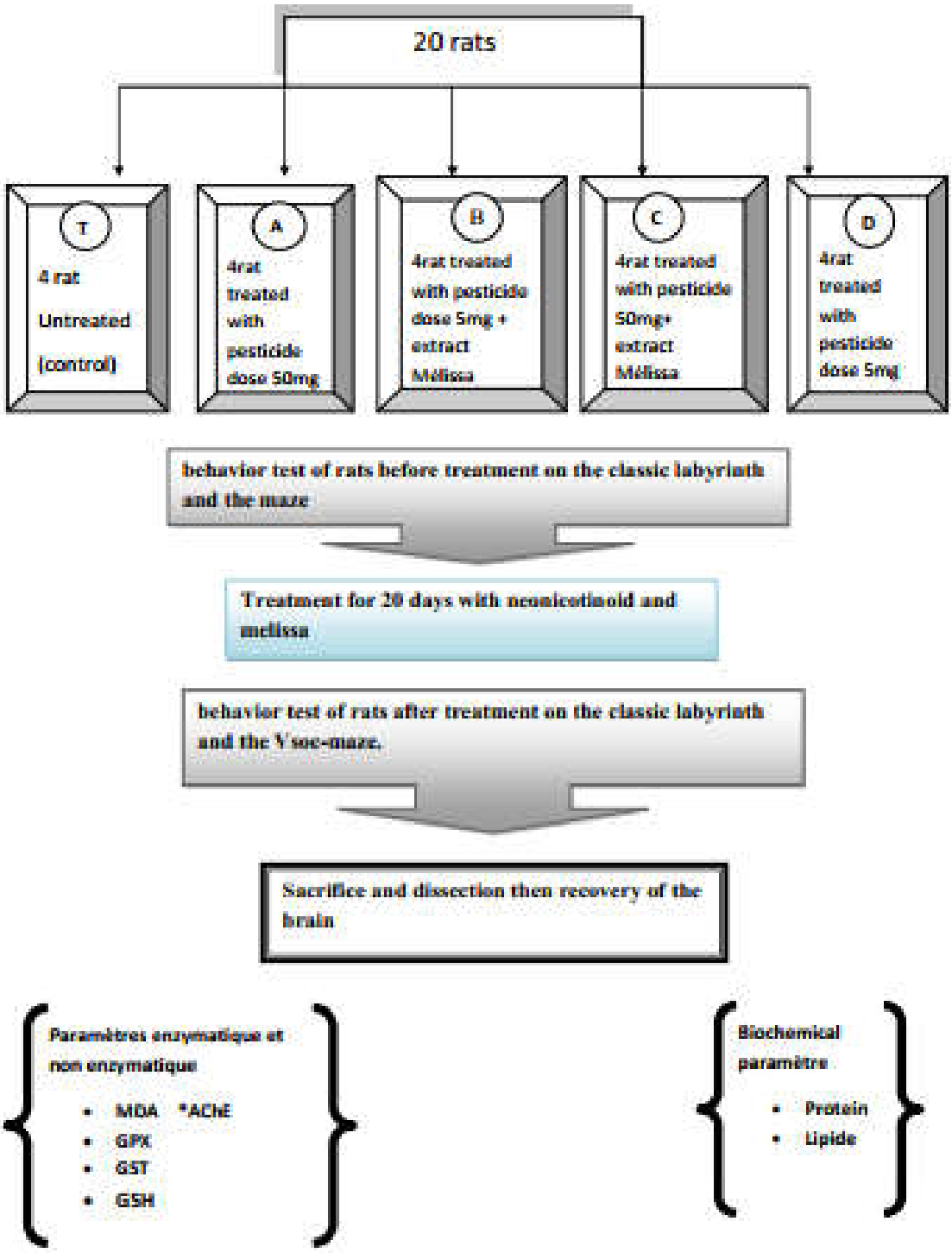


Figure 31: schematic of whole experimental work

2.6. Study of the cellular and molecular integrity of neurocytes

After the sacrifices of the rats we recovered the brains, we rinse them ,then we measure them with a precision balance; in 4 to 6 hours maximum we prepare the mitochondrial used to assess swelling, permeability and breathing, mitochondrial

2.6.1 Preparation of mitochondrial suspensions

The mitochondria are extracted according to the method described by **(Rusten and *al*., 1994)** it is a purification by differential centrifugation. Briefly, after decapitation of the rats, the brains are quickly removed and immersed in a TSE buffer. (10mM tris , 250 mM sucrose, 0,1 mM EDTA, pH 7,2 to 4C°).

- Nerve tissue is finely cut and posterized in 3.5ml of TSE, which allows the destruction of cells and the release of mitochondria,
- The recovered homogenate is centrifuged at 10,000 rpm for 10 min,
- thus allowing the removal of large cellular debris.
- The recovered pellandis centrifuged a second time at 10,000 rpm for 10 min
- Supernatants from two centrifugations are recovered and centrifuged at 14,000 rpm at 4 ° C. for 10 min
- The pellandobtained is resuspended in 1 ml of TSE and centrifuged at 14,000 rpm for 10 min..
- The pellandfrom this last centrifugation is resuspended in 1 ml of TS buffer (250 mM sucrose, 50 mM tris, pH 7,2 to20 C°) is centrifuged for 10 min at 14,000 rpm.
- The final pellandconsisting of mitochondria, and is recovered in 500 µl of TS buffer to obtain the fresh mitochondrial suspension,
- a fraction of which will be used directly in the evaluation of the structural and functional integrity of the mitochondria,
- the rest is kept at -80 ° C for further dosing.

2.6.2 Preparation of the mitochondrial matrix

The mitochondrial suspension is frozen-thawed 6 to 8 times with accumulated and repeated poteration, in order to burst the mitochondria.

- After centrifugation for 10 min at 9600 rpm, the supernatant is used as the source of the stress parameters **(Lahouel and *al*.,2015)**

2.6.3 Assessment of swelling, patency and mitochondrial respiration

According to the method of (**kristal and *al*.,1996)**, we carried out the estimation of mitochondrial permiablite based on the rate of Ca++ crossing their membranes, this permeability followed by an increase in the size of the mitochondria detected at a wavelength of 540 nm for 3 min and every 30 seconds , respiration was estimated using an axygraph (Hansatech ®) according to the method described by (**Rouabhi and *al* .,2006;2009)**

3. Methods for the determination of biochemical parameters

3.1. Metabolic parameters

3.1.1. Extraction and determination of metabolites

The extraction of the various metabolites was carried out according to the method of **Duchatear and Flarkin** the main steps are summarized in the **(figure 32)**below , Control and treated samples are ground using a magnetic mill in trichloroacetic acid (TCA) to20% (200 mg of organ 1 ml of TCA). After a first centrifugation (5000 rpm, pendant 10min the In pellandI, on adding 1 ml of ether / chloroform mixture (1V / 1V) and after a second centrifugation (5000 rpm, in 10 min), on obtaining the supernatant II and the pellandII, the supernatant II will be used for the lipid assay (**Goldsworthy and *al*.,1972)** and pellandII, dissolved in NaOH (0.1 N), will be used for the determination of proteins, according to **(Bradford., 1976).**

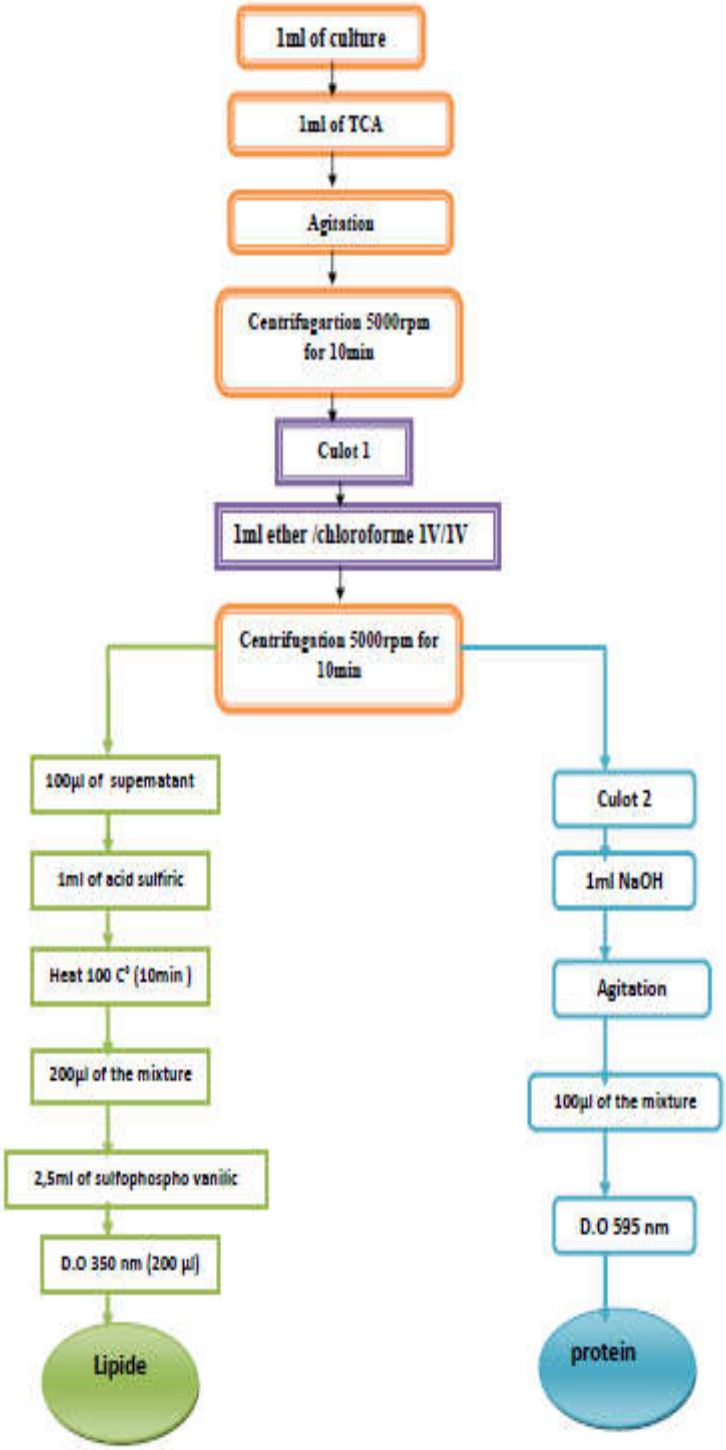


Figure32: method of extraction and determination of the main biochemical constituents (lipid and protein)

3.1.2. Assay methods

3.1.2.1. Dosage of total lipids

- Total lipids were determined according to the method of **(Goldsworthy and *al*., 1972)** using the sulfo-phospho-vanillinic reagent (0.38 g of vanillin, 195 ml of 85% orthophosphoric acid and 55 ml of distilled water) and a stock lipid solution (2.5 mg / ml) as standard.

● Adding 1 ml of sulfuric acid (98%), after stirring, the tubes are heated in a water bath (100 ° C for 10 min); 200 µl of each tube are then with drawn and 2.5 ml of reagent are added;

The absorbances were read after 30 min of darkness at a wavelength of 530 nm.

3.1.2.2. Dosage of total proteins

The assay of the proteins is carried out according to the method of (**Bradford.,1976).** Which uses the brilliant blue of coomassie (BBC) as reagent. This reveals the presence of proteins in the blue dyes And (B.S.A) as standard. The calibration range was carried out using a stock solution of bovine serum albumin (BSA). (1 mg / ml) and the B.B.C (storage about 21 days at 4 ° C) which is prepared as follows:

- 100 mg of BBC + 50 ml of ethanol Agitation for two hours;

● 100 ml of orthophosphoric acid are then added and the whole is made up to 1000 ml with distilled water;

● The protein assay was carried out in an aliquot fraction (100 ml);

● The absorbances were read in a spectrophotometer at a wavelength of 595nm.

3.2. Oxidative stress parameters

Which includes enzymatic and non-enzymatic parameters.

3.2.1. Non-enzymatic biomarkers

3.2.1.1 Dosage of Malondialdehyde (MDA)

MDA is one of the products terminated during the breakdown of polyunsaturated fatty acids (PUFA) mediated by free radicals.

Principle

Malondialdehydes (MDA) are assayed according to the method of (**Eserbauer and *al*.,1992)** This method is based on the colorimetric measure of the reaction from the acid thiobarbituric (TBA) and the malondialdehyde (MDA) in an acidic and hot medium (100 ° C) giving a red brown product whose color is obtained at a wavelength of 530 nm.

Experimental protocol

Prepare the homogenates from 200 mg of organ with TP homogenization buffer (pH 7.4);

Centrifuge at 3000 rpm for 10 min;

Take 375 µl of supernatant;

Add 150 µl of TBS solution buffer (50 mM Tris, 150 mM NaCl pH 7.4);

Add 375 µl of TCA-BHT solution (TCA 20%, BHT 1%);

Agitator and centrifuge at 1000 rpm for 10 min;

Take 400 µl of supernatant;

Add 80 µl of 0.6 M HCl;

Add 320 µl of Tris-TBA solution (Tris 26 mM, TBA 120 mM);

Mix and incubate in a water bath at a temperature of 80 ° C for 10 min;

Read The optical density at $\lambda = 530$ nm.

- The absorbance is directly proportional to the amount of MDA formed, thus giving an accurate assessment of the peroxidized lipids.

3.2.1.2 Glutathione (GSH) dosage

Principle of the method

The principle of this assay is based on the measurement of the optical density of 2-nitro-5-mercapturic acid. This last result of the reduction of 5,5'-dithio-2-nitrobenzoic acid (Ellman reagent or DTNB) by the groups (-SH) of glutathione. Once prepared, the homogenate must undergo deproteinization with 0.25% sulfosalicylic acid to protect the (-SH) groups from (**weckbeker and Cory glutathione.,1988).**

Experimental Protocole

Prepare the homogenates from 1 ml of culture with EDTA phosphate buffer (0.02M);

Take 0.8 ml of the targandhomogenate and add 0.2 ml of a 0.25% sulfosalicylic acid solution (SSA);

Shake the mixture and landit hang for 15 min in an ice bath;

Centrifuge at the speed of 1000 rpm for 5 min;

Take 0.5ml of supernatant;

Add to the mixture: 1 ml of Tris-EDTA buffer (0.02 M EDTA, pH 9.6), 0.025 ml of DTNB and 0.5 ml of the supernatant.

Landstand for 5 minutes at room temperature for color stabilization. The colorimetric reaction develops instantly;

Measure the absorbances at 412 nm against the blank.

3.2.2. Enzyme biomarkers

3.2.2.1. dosage of Glutathione peroxidase (GPx) activity

The enzymatic activity of glutathione peroxidase (GPx) was tested by the method of (**flohe and gunzler.,1984)**This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of the reduced glutathione (GSH), this last is in (GSSG) under the influence of GPx according to the following reaction:

$$H_2O_2 + GSH \xrightarrow{GPx} GSSG + 2H_2O_2$$

Experimental Protocol

Prepare the homogenates from 200 mg of organ with TP homogenization buffer (pH8);

Centrifuge at 3000 rpm for 10 min;

Take 0.2 ml of supernatant;

Add 0.4 ml of GSH (0.1 mM);

Add 0.2 ml of TBS solution buffer (50 mM Tris, 150 mM NaCl, pH 7.4);

Incubate in a water bath at 25 ° C, for 5 min;

Add 0.2 ml of H2O2 (1.3 mM) to initiate the reaction, left to act for 10 min;

Add 1 ml of TCA (1%) to stop the reaction;

Put the mixture in an ice bath for 30 min;

Centrifuge for 10 minutes at 3000 rpm;

Take 0.48 ml of supernatant;

Add 2.2 ml of TBS solution buffer;

4 Add 0.32 ml of DTNB (1 mM);

Mix and after 5 minutes; read the optical densities at 412 nm.

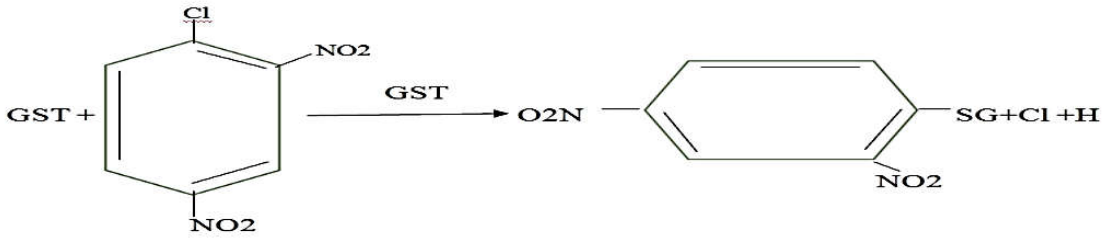
3.2.2.2 Dosge of Glutathione S-Transferase (GST) activity

The glutathione S-transferases developed to a family of essentially cytosolic multifunctional enzyme, applied in transport and intracellular biosynthesis, they catalyze conjugation reactions between an endogenous peptide, glutathione, and reactive molecules containing electrophilic sites..

The measurement of glutathione S-Transferase (GST) activity is determined according to (**Habig and *al*., 1974)** method; it measures the kinetics of formation between a model substrate, chlorodinitrobenzene (C-DNB) and glutathione

It is based on the conjugation reaction between TPS and a substrate, CDNB (1-Chloro2, 4 di nitrobenzene) and a co-actor glutathione (GST), the conjugation leads to the formation of a new molecule.

1-S-Glutathionyle 2-4Di nitrobenzene used to measure the activity of GST according to the following reaction:



The value of the optical density obtained is directly proportional to the amount of conjugate formed itself linked to the intensity of the TPS activity.

To do this, we have the following steps:

Homogenization with 1 ml of phosphate buffer (0.1 M, pH 06).

The homogenate is centrifuged at 14,000 rpm for 30 min and the recovered supernatant will serve as the source of enzymes.

The assay consists in reacting 200 µl of supernatant with 1.2 ml of CDNB (1 mM), GSH (5 mM) mixture [20.26 mg CDNB, 153.65 mg GSH, 1 ml ethanol, 100 ml phosphate of buffer (0.1 M, pH 06)].

The absorbances are read for one minute and every 15 seconds at a wavelength of 340 nm against a blank containing 200 µl of distilled water replacing the amount of supernatant.

The absorbance is read at 340 nm after 30 s in 3 min

3.2.2.3. Dosage of catalase activity (CAT)

- The spectrophotometric measurement of catalase activity (CAT) is carried out according to the method of (**Cakmak and Horst.,1991)**. The decrease in absorbance is recorded for three minutes by a spectrophotometer for a wavelength of 240nm and a coefficient molar line extinction = 39400 $\mu M^{-1}.cm^{-1}.L$, for a final volume of 3 ml; the reaction household contains:100µl of the crude enzyme extract, 50 µl of hydrogen peroxide H□O□ at 0.3% and 2850 µl of phosphate buffer (50 mm, pH 7.2).the hydrogenation calibration is carried out in absence of the enzyme extract. the reaction triggered by the addition of hydrogen peroxide.

3.2.2.4. Determination of acetylcholinesterase (AChE)

The most common AChE acetylcholinesterase assay **(ellman and and *al.*, 1961)** is to provide the enzyme with a substrate, lectylecholine, the hydrolysis of which releases thiocholine and acetic acid. the sample is homogenized in 1 ml of detergent

solution (38.03 mgethylethylene glycol tris- β-aminoethyl ether NN N'N, 1 ml triton X100% 5.845g NaCl , 80 ml tris buffer 10 mm) using an ultrasonic homogenizer and then centrifuges at 5000 rpm for 5 min, the supernatant is used immediately for the measurement of AChE activity. the AChE assay steps are as follows:

- ✓ 100 µl of supernatant + 100 µl of DTNB (0.1 M, ph = 8) (39.6 mg of DTNB, 15 mg CO_3 Na, in 1 ml tris (0.1 M, ph7) and 1 ml of tris buffer (0.1 M , ph 7)
- ✓ After 5 minutes of rest necessary to exhaust the spontaneous reaction, 100 µl of acetylthiocholine substrate (118mg Ach without 5ml distilled water)
- ✓ The densities are read at 412nm every 4min for 20min.

4. Statistic study:

- ✓ The results are obtained from a test with a criterion present in the form (mean plus / minus standard deviation) the results obtained are represented by histograms.
- ✓ We determined the statistical parameters for each experimental batch. Data were analyzed by analysis of variance with P significance rate and compared to controls by the test of Dunnett. These calculations were carried out using MINITAB 17.01 software and Exel 2013.
- ✓ the comparison result as follows:
- ✓ • $p > 0,05$ = the difference is not significant,
- ✓ • (*) $0,05 > P > 0,01$ = the difference is significant,
- ✓ • (**) $0,01 > P > 0,001$ = the difference is highly significant,
- ✓ • (***) $P < 0,001$ = the difference is very highly significant.

Chapter 02 :

Result

1. Result:

1.1.Growth parameters:

1.1.1. Action of Imidacloprid and Melissa sp and the imidacloprid / Melissa sp combination on body growth in rats treated for 20 days

Results of the evaluation of the growth parameters in terms of body weight, weight gain and relative weight during the 90 days of treatment of the different groups of animals with imidacloprid 5mg, 50mg, and imidiacloprid /Melissa extract.

1.1.1.1. Weight gain:

The results of the weight gain assessment **(Figure 33)** show a highly significant reduction ($p \leq 0.01$) in weight gain in the lots treated with imidiacloprid (5mg) and imidiacloprid 50mg / M. extract compared to the control lot . These results also show a very highly significant decrease ($p \leq 0.001$) in the rats treated with imidiacloprid (50 mg) compared to the control group. On the other hand, there is an improvement in weight gain each time that the midiacloprid is combined with the M. extract like the batch treated with imidiacloprid (5mg) / the M. extract.

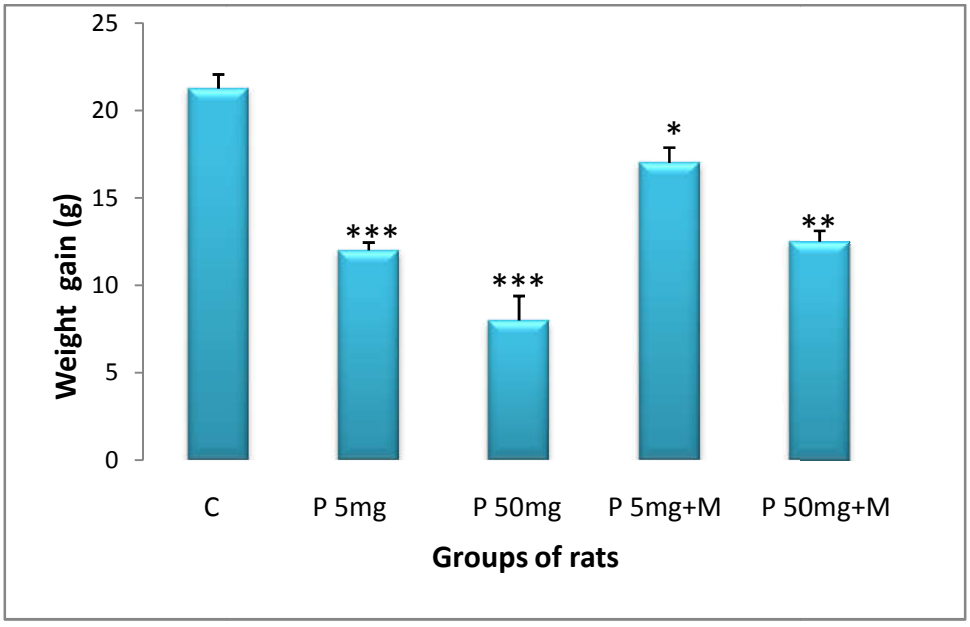


Figure33: Evolution of body weight gain (GP) in control rats treated after 20 days of treatment with imidiacloprid (5mg / 50mg) and imidiacloprid(5mg/50mg) / melissa extract

1.1.1.2. Relative weight of the brain (RBW):

The results obtained following the evaluation of the RZ, only show a non-significant increase (P> 0.05) in the relative weight of the brain in the group treated with imidiacloprid (50 mg) in comparison with the control group. **(figure 44)**

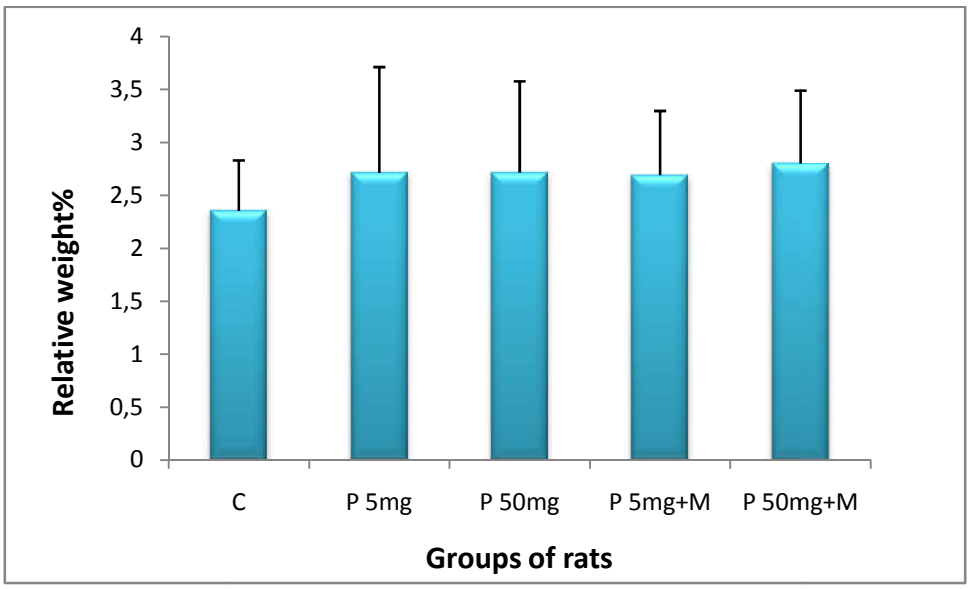


Figure34: Evolution of the relative weight of the brain (RWB) in rats treated for 20 days with imidiacloprid (5mg/50mg) and imidiacloprid (5mg/50mg)/Melissa extract

1.2 Effects of imidacloprid and Melissa extract on the parameters of membrane permeability and mitochondrial swelling in the total brain in rats:

1.2.1 Effect of imidacloprid and imidiacloprid/Melissa extract on mitochondrial swelling:

Results regarding the effect of imidacloprid and the extract on mitochondrial swelling in the overall brain in the figure.

The results of the mitochondrial swelling assessment are illustrated in the figure, which shows a very highly significant increase ($p \leq 0.001$) in the rate of mitochondrial swelling in the overall brain in the imidiacloprid treated groups (p50mg) compared to rats witnesses. A very significant increase ($p \leq 0.01$) in the rate of mitochondrial swelling is also recorded in the batch treated with imidiaclprid (p5mg) compared to the control rats. **(Figure35)**

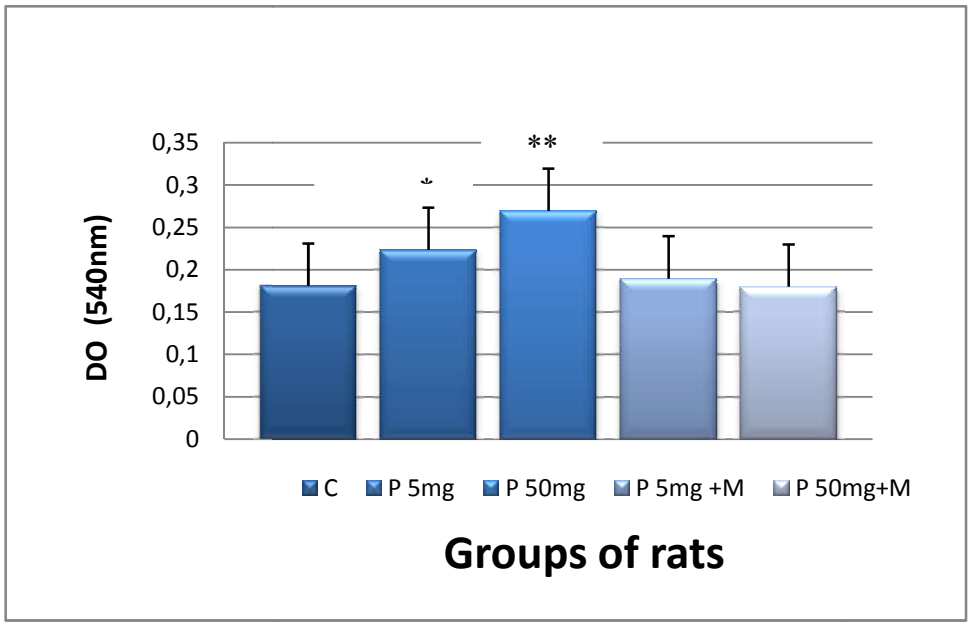


Figure35: Variation in mitochondrial swelling of total brain cells in rats treated for 20 days with imidiacloprid(5mg/50mg) and imdiacloprid(5mg/50mg) / M. extract

1.2.2. Effect of imidacloprid and imidacloprid / Melissa extract on mitochondrial membrane permeability:

A very highly significant increase ($p \leq 0.001$) in mitochondrial permeability is recorded in the overall brain in the batches treated with imidiacloprid (P5mg / 50mg) compared to the control group **(Figure36).**

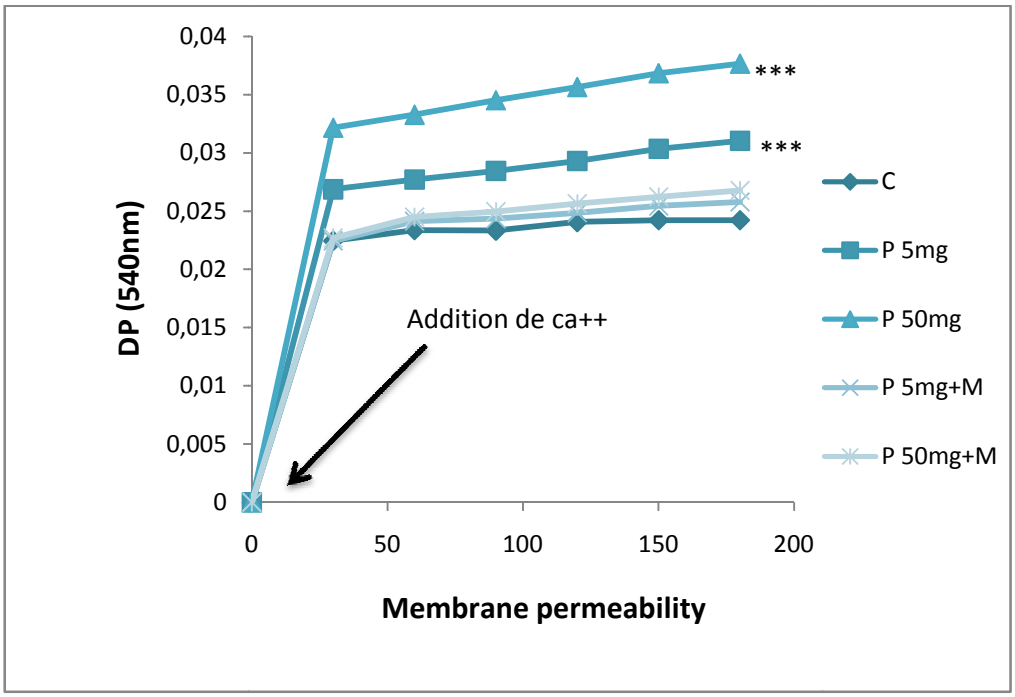


Figure36: Variation in mitochondrial membrane permeability in the total brain in rats treated for 20 days with imidiacloprid (5mg / 50mg) and imdiacloprid (5mg / 50mg) / M. extract

1.3. Effect of imidacloprid and imidacloprid / Melissa extract on biochemical parameters in the brain in rats:

1.3.1. Effect on protein levels:

Variation in protein content in control rats treated with imidiacloprid and imidiacloprid / Melissa extract are shown in the figure.

The results presented in the figure show that the protein level of the batches treated with imidiacloprid (5mg) has increased in a very significant way ($p \leq 0,001$) compared to the witness. There is also a very highly significant increase in the batch treated with imidiacloprid (50mg) ($p \leq 0,0001$). but the batch treated with imidacloprid (5mg) / Melissa extract, there is almost no change compared to the controls, We also note that there is a non-significant increase in the batch treated with imidiacloprid / extra Melissa compared to controls.**(Figure37)**

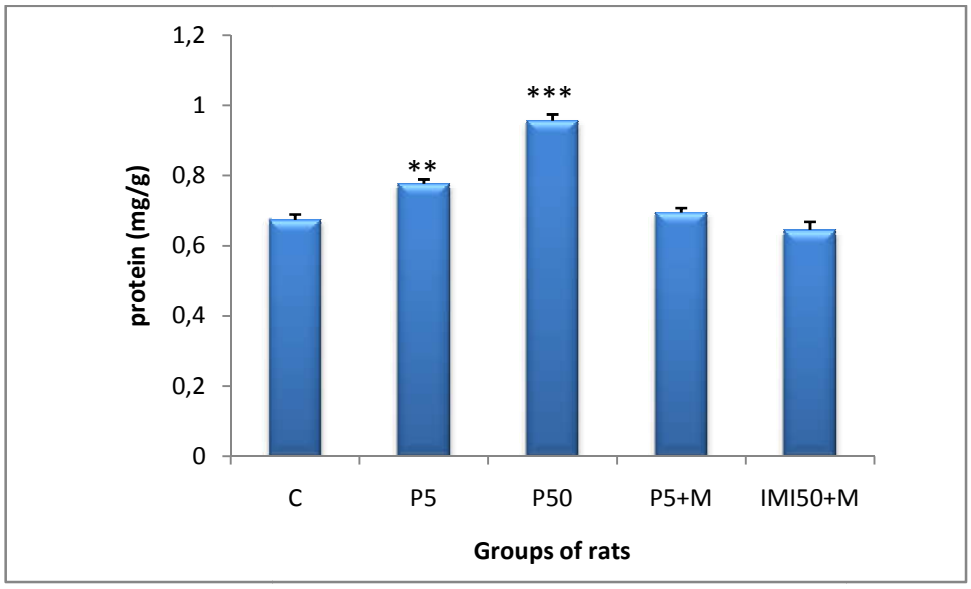


Figure37: Variation in protein levels in the brain of control and treated rats after 20 days of treatment with with imidiacloprid (5mg / 50mg) and imdiacloprid (5mg / 50mg) / M. extract.

1.3.2. Effect on lipid levels:

Variation in lipid content in control rats treated with limidiacloprid and imidiacloprid / Melissa extract are shown in the figure.

We note that the lipid level in the batches treated with imidiacloprid (5mg) decreased significantly compared to the control batches ($p \leq 0,005$), there is also a very highly significant decrease in the lot treated with imidacloprid (50 mg) ($p \leq 0.0001$). but after the addition of the

Melissa extract (the combination imidacloprid / Melissa extract) we notice a very insignificant increase compared to the control.**(Figure38)**

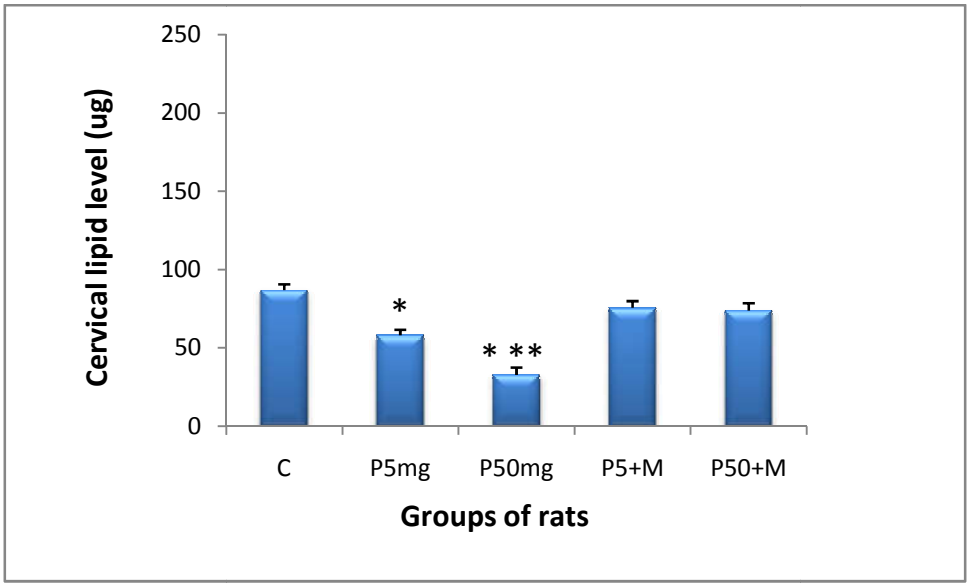


Figure38: Variation in lipids levels in the brain of control and treated rats after 20 days of treatment with with imidiacloprid (5mg / 50mg) and imdiacloprid (5mg / 50mg) / M. extract.

1.4. Imidiacloprid induced oxidizing stress: effect protective ofMelissa extract

1.4.1 Effect on the rate of malondialdehyde (MDA):

According to the results presented in the figure, there is a very highly significant increase (P = 0.000) in the rate of in rats treated with imidiacloprid (50mg) and a highly significant increase ($p \leq 0.001$) in rats treated with imidiacloprid (5mg), this increase is regulated after the addition of the Melissa extract compared to the controls.**(Figure39)**

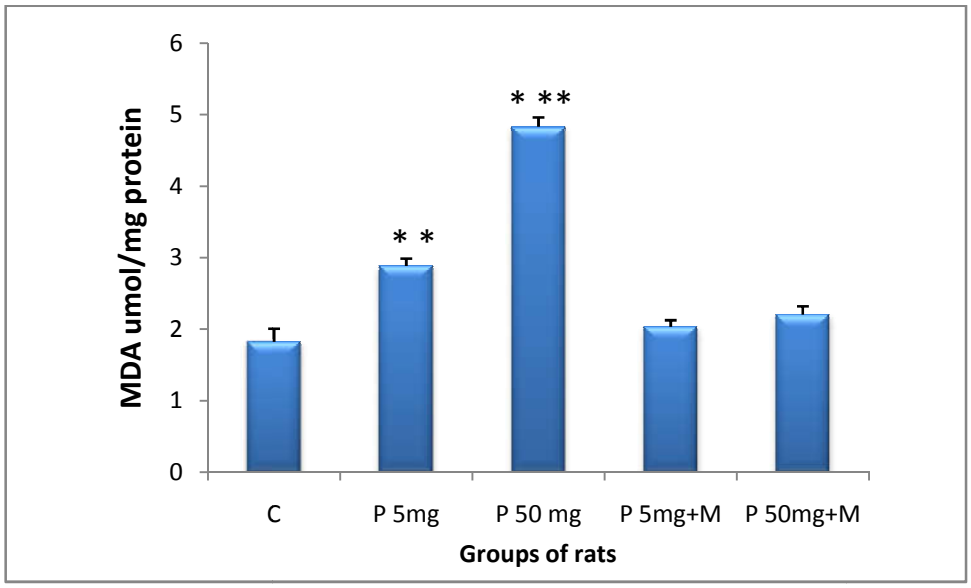


Figure39: Variation in MDA level (µmol / mg protein) in the brain of control rats and treated rats after 20 days with imidacloprid(5mg/50mg) and imidacloprid(5mg/50mg)/M.extract

1.4.2. Effect on GSH level:

The variation in GSH level in the treated and control rats is presented in the figure.

The results obtained show a significant decrease ($p \leq 0.005$) in the batches treated with imidiacloprid (5mg) and a very highly significant decrease in the batch treated with imidiacloprid (50mg) compared to controls.this reduction is neutralized after the addition of the Melissa extract in a significant manner.**(Figure40)**

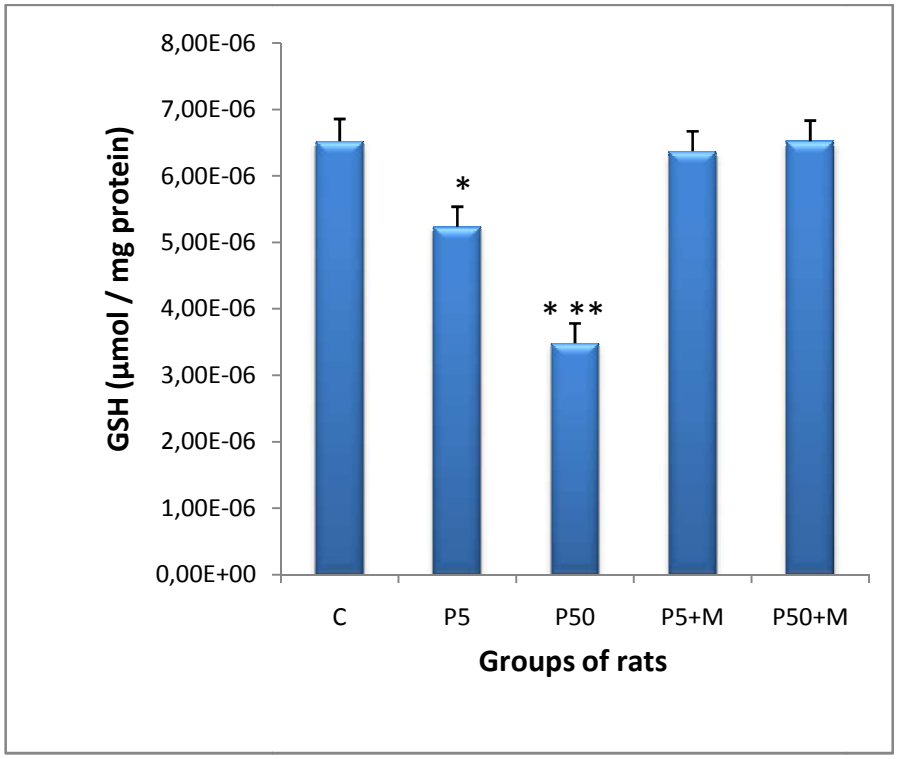


Figure40: Variation in the level of GSH (µmol / mg protein) in the brain of control and treated rats after 20 days of treatment with imidacloprid(5mg/50mg) and imidacloprid(5mg/50mg)/M.extract

1.4.3. Effect on variations in GST activity:

The figure shows the variation of the enzymatic activity of GST in the liver in the control and treated rats.

Our results show a significant increase ($p \leq 0.005$) in the batch treated with imidiacloprid (5mg) but there is a highly significant increase in the batch treated with imidiacloprid (50mg) compared to the controls. On the contrary, during the addition we observe a neutralization compared to the controls.**(Figure41)**

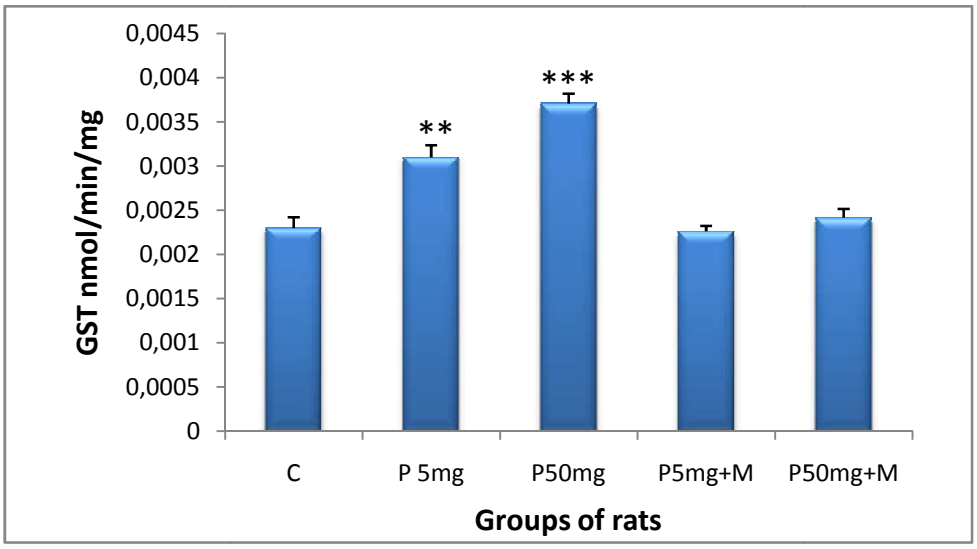


Figure41:GST enzymatic activity in the brains of control and treated rats after 20 days of treatment with imidacloprid(5mg/50mg) and imidacloprid(5mg/50mg)/M.extract

1.4.4. Effect on GPx activity:

The figure shows the variation in the enzymatic activity of GPx in the brains of control rats treated with imidiacloprid (5 mg / 50 mg) and the Melissa extract in combination with imidiacloprid.

Our results show a non-significant decrease compared to the control in rats treated with imidiacloprid (5mg), but there is a very significant decrease ($p \leq 0.005$) in rats treated with imidiacloprid (50mg). this reduction is neutralized after the addition of the Melissa extract.**(Figure42)**

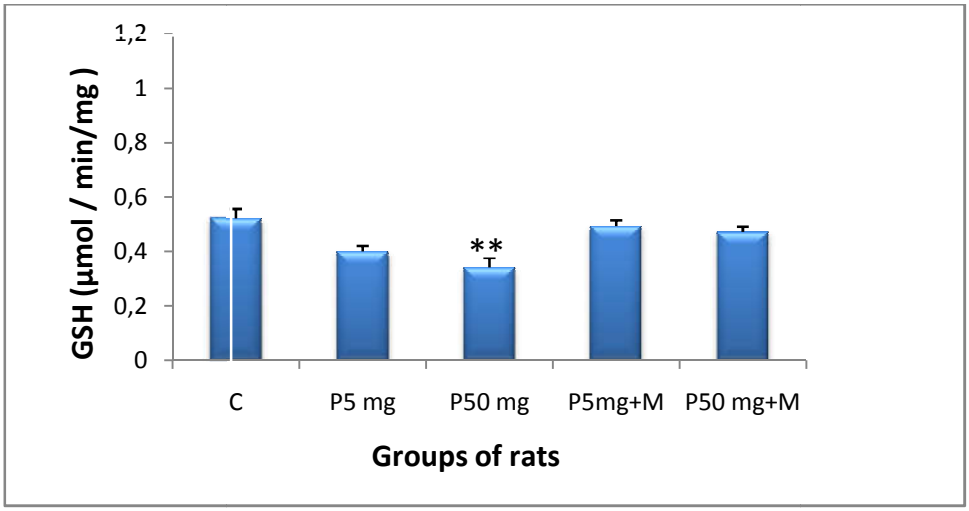


Figure42: GPX enzymatic activity (µmol / min / mg protein) in the brain of control and treated rats after 20 days of treatment with imidacloprid(5mg/50mg) and imidacloprid(5mg/50mg)/M.extract

1.4.5. Effect on CAT activity:

Our results show a significant decrease ($p \leq 0.005$) compared to the control in rats treated with imidiacloprid (5 mg), but there is a very high significant decrease ($p \leq 0.0001$) in rats treated with imidiacloprid (50 mg). this reduction is neutralized after the addition of Melissa extract.**(Figure43)**

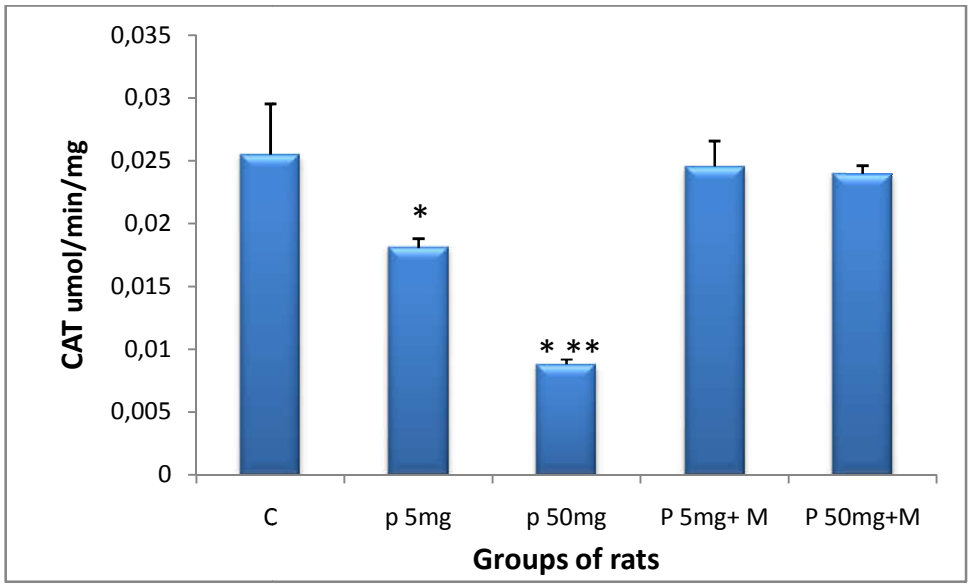


Figure43: Variation of the enzymatic activity of catalase in the brain of control and treated rats for 20 days of treatment with imidacloprid(5mg/50mg) and imidacloprid(5mg/50mg)/M.extract

1.5. Effect on acetylcholinesterase activity:

A non-significant decrease in the activity of AchE is recorded in the brain in the groups treated with imidiacloprid (5 mg) and a significant decrease ($p \leq 0.005$) in the activity of this enzyme is also observed in the batch. treated with imidiacloprid (50mg) compared all to the control batch. On the other hand, no significant difference was observed in the rest of the groups in comparison with the control **(Figure44)**

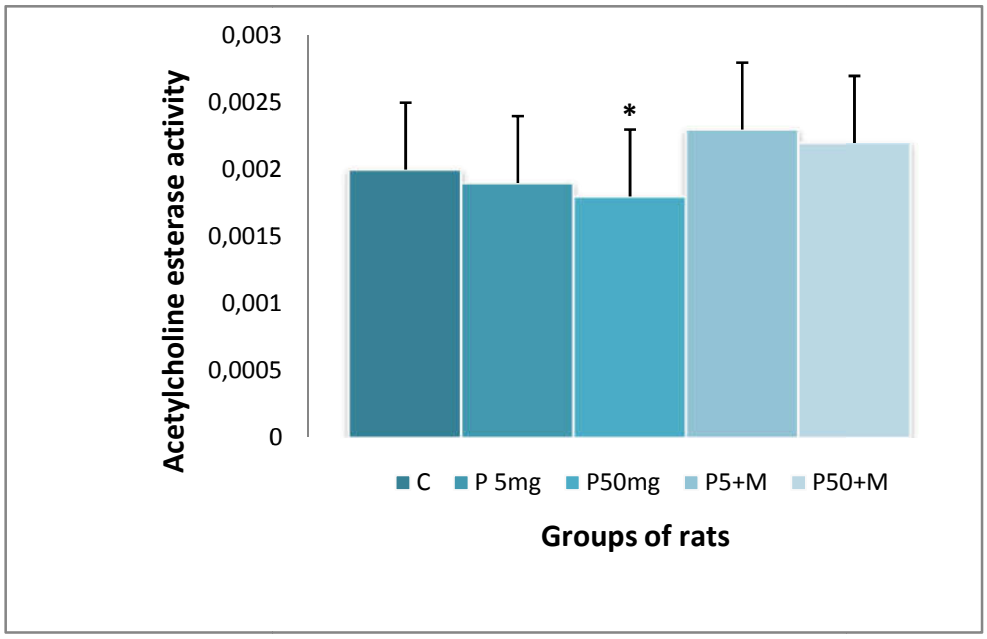


Figure44: Acetylcholinesterase activity in the brain in control and treated rats after 20 days of treatmen with imidacloprid(5mg/50mg) and imidacloprid(5mg/50mg)/M.extract

1.6. Effects of imidacloprid and Melissa extract on neuro-behavioural in rats:

1.6.1. Classic Labyrinth Test:

The results obtained following the examination of this test on animals are illustrated in the figure. Statistical analysis of these results showed a very highly significant increase ($p \leq 0.001$) in arrival time (fig-A) and also a very highly significant increase in exploration time (fig. 43-B) in batches treated with imidiacloprid (5 / 50mg) when compared to controls. Also, a significant increase in exploration time was also observed in the batch treated with imidiacloprid (50 mg) / extract M compared to the controls. Also, an increase but not significant in exploration time was observed in the batches treated with imidiacloprid (50mg) / M extract. There is a significant increase in batches treated with imidiacloprid (5 / 50mg) / M extract of arrival time compared to controls.**(Figure45)**

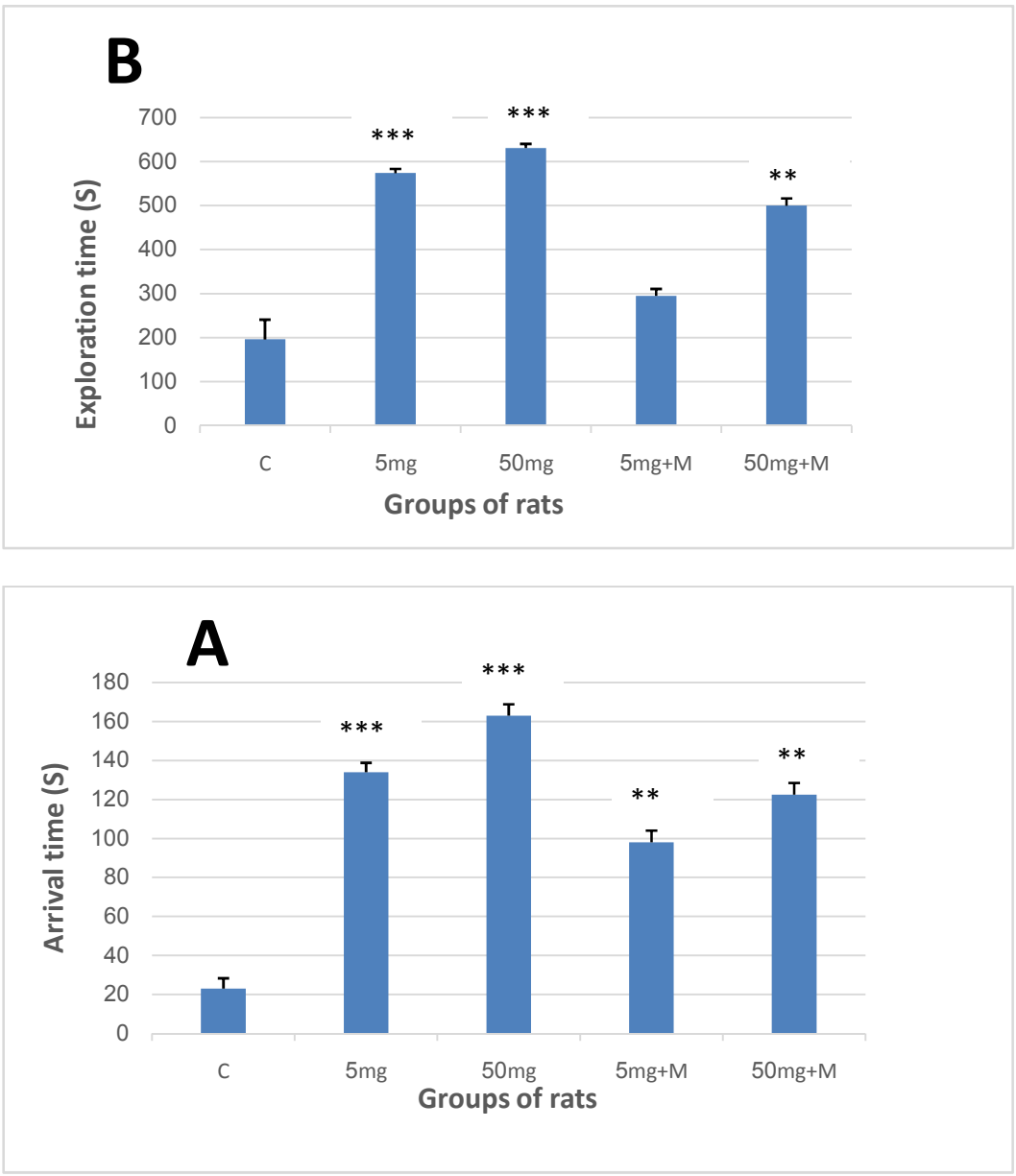


Figure45: : Variation in classic Labyrinth test in groups of rats treated with imidacloprid (5mg/50mg) and imidacloprid(5mg/50mg)/ Melissa extract for 20 days. (A): Time of arrival at the final point in the labyrinth. (B): Time to explore the middle of the labyrinthe

1.6.2. the Vsoc-maze:

The results obtained from the maze vsoc neurobehavioural examination are mentioned in the table below (table 10)

For the 1st test there is a random movement and an unspecified time spent for all the lots tested the meaning is not important.

For the 2nd test, the batch treated with the 5 mg dose of pesticide, showing a result in the 15th day, otherwise the rats treated with a dose of 50 mg of pesticide mentioned them within 10 days.

For the 3rd test, the batch with the 5 mg dose of imidacloprid having a behavior appeared in the 15 th day, but the rats treated with 50 mg, mentioning a highly significant increase in the outcome within 10 days.

On the other hand, the batch treated with the 5mg dose added to a treatment with the extra melissa records a significant decrease during the whole period of exament, on the other hand, the differnce was observed on rats treated with the dose of 50mg of pesticide + a dose of lemon balm within 15 days.

Table08: Represents the time to be explored in the vsoc labyrinth chambers by the rats treated during the behavioral examination

STAGE 1	CT	DOSE 5	DOSE 50	DOSE 5+M	DOSE 50+M
RM 01	115 s	142,5s	125s	148s	156s
RM 02	134,75s	124,25s	123,75s	138s	134,5s
STAGE 2	CT	DOSE 5	DOSE 50	DOSE 5+M	DOSE 50+M
RM 01	244,25s	151s	96s	189,3s	161s
RM 02	29,25s	155s	199s	100s	140s
STAGE 3	CT	DOSE 5	DOSE 50	DOSE 5+M	DOSE 50+M
RM 01	33,25s	156,5s	190,5s	118s	145s
RM 02	245s	138s	92s	205s	155s

M :Melissa

CT:control

RM:Room

Chapter 03 :
Discussion

1.Discussion:

We tried in the present work to evaluate the effects of Imidacloprid on enzymatic and biochemical parameters in the brain of wistar rats as a biological model, so we looked for neurotoxic effects at the mitochondrial level, we also interested the protective effect of meliss against the neurotoxicity induced by Imidacloprid. For a better understanding of the mechanisms and causes of toxicity, the results obtained are analyzed and discussed based on several studies.

Under our experimental conditions, the percentage of mortality in rats is zero.

1.1 Effects of Imidacloprid and Meliss on Global Growth Parameters

The results of the evaluation of the weight parameters suggest that the administration of Imidacloprid causes a significant decrease in the body growth of the different groups of rats. This reduction can be translated by the disturbance of cellular metabolism under the effect of the oxidative stress generated by the ROS observed in this study, as well as by other chemical mediators such as certain proinflammatory cytokines that the organism can release after the toxic effects. pesticides**(Carole and *al*.,2011 ; Viviana, 2015),** who reported a reduction in food consumption in male rats that occurred with subchronic toxicity. Furthermore, the use of meliss has shown an improvement in these animal weight parameters. This could be the consequence of its antioxidant power by normalizing intracellular redox homeostasis and restoring the psychic state of animals **(Cliona and *al*., 2011 ; Toumi and *al*., 2016).**

1.2. Effects of Imidacloprid and Meliss on Metabolites

Membrane lipids, which are particularly rich in polyunsaturated fatty acids (PUFA), represent a privileged targandfor radical aggression. The more the fatty acid is rich in double bonds, the more it is peroxidable, that is to say degradable by an oxidative process harmful to the cell. Lipids also are the seat of lipid peroxidation. Their oxidation leads to membrane disorganization with changes in fluidity and permeability **(Hiltenbr and *al.,* 1999; Durand and *al*., 2013).**

Treatment with Imidacloprid at a different dose (5 and 50 mg / kg / D) for 20 days in rats is induced significant metabolic alterations in the brain by very highly significant reduction in total lipid content in comparison with the control, this decrease can be stimulated by free radicals in case of oxidative stress generated by Imidacloprid.

On the other hand, according to the results obtained when using meliss as a cytoprotective molecule, it turns out to have improved the homeostasis of the biochemical parameters studied in this present work.

Also increasing protein levels after sub-acute exposure of rats to Imidacloprid translates the synthesis of enzymes and defense peptides against the homeostatic imbalance of oxidative stress **(Anadn and *al*., 1991; Benbouzib, 2012; Rouabhi and *al*., 2015).**

On the other hand, according to the results obtained when using meliss as a cytoprotective molecule, it turns out to have improved the homeostasis of the biochemical parameters studied in this present work.

1.3. Effects of Imidacloprid and Melissa on oxidative stress parameters

Oxidative stress is one of the main mechanisms of toxicity associated with a variety of xenobiotics in the environment, among which are pesticides and phytosanitary products **(Lauvverys and *al*., 2007 ; Lukaszewicz, 2008 ; Michael and *al*., 2016).**

Biochemical and enzymatic parameters in organisms exposed to toxic contaminants have been used as biomarkers and can be an important diagnostic tool for assessing exposure and the effects of xenobiotics **(Forbes and *al*., 1997).**

1.3.1. Malondialdehyde (MDA)

MDA is the main compound resulting from the formation of various aldehydes toxic to the organism following the oxidative degradation of polyunsaturated fatty acids in cell membranes **(Maiza and *al*., 2011).**

In this study, treatment with the different doses of Imidacloprid in Wistar rats during this period results in a very highly significant increase in the level of MDA in the two experimental batches compared to the control. This experimental finding is entirely in line with the work done by **(Chakroun and *al*., 2016; Beghoul and *al*., 2017)** who reported an increase in MDA as an indicator of ROS-mediated lipoperoxidation under the effect of these pesticides .

1.3.2. Glutathione (GSH)

GSH is a tripeptide well known to be an element of the first line of defense against stress and therefore considered an essential compound which maintains cell integrity thanks to its reducing property and its active participation in cell metabolism**(Sauer, 2014 ; Aoun and Tiranti, 2016).**

Some of the important roles of glutathione are the reduction or inactivation of ROS by the formation of glutathione disulfide (GSSG) and the conjugation of reduced glutathione (GSH) for the elimination of xenobiotics **(Di-Monte and Lavasani, 2002 ; Arora and *al*., 2016 ; Rjeibi, 2016).**

The results obtained from the evaluation of GSH after the sub-acute exposure of animals to pesticides, showed a significant decrease in its content in the brain.

1.3.3. Effect on GPx activity

GPx, antioxidant enzymes, are one of the main lines of defense against attacks produced by free oxygen radicals. The GPx family is subdivided into four subfamilies: the most abundant in animals is the cellular GPx. Localized mainly in the cytosol, its role is to trap H2O2 (whereas the H2O2 produced in peroxisomes is trapped by catalase located in these organelles). The membrane GPx (phospholipid hydroperoxide GPx) has a role in the protection of biomembranes against lipid peroxidation. There is also extracellular (plasma) GPx and gastrointestinal GPx which also inhibit the production of free radicals **(Fournier, 2005).**

A very highly significant reduction in GPx levels in rats treated with (5.50 mg / kg / day) after a 20-day exposure to Imidacloprid.

which confirms the state of oxidative stress induced by these pesticides in the brain tissue. Same results provided by other work on the impact of pyrethroids and neonicotinoids on the brain of the animal organism**(Halliwell, 2006 ; Chakroun and *al*., 2016 ; Beghoul and *al*., 2017).**

1.3.4. Effect on GST activity

Glutathione transferases (GSTs), also known as glutathione S-transferases) are major phase II detoxification enzymes found primarily in the cytosol. In addition to their role in catalysis, the conjugation of electrophilic substrates to glutathione (GSH), these enzymes also perform a range of other functions. They have peroxidase and isomerase activities **(Sheehan and *al*., 2001).** They play an important role in the detoxification of Xenobiotics **(klibandand *al*., 2009).**

The biochemical results of the group treated with Imidacloprid revealed a very highly significant increase ($P \leq 0.01$) in the enzymatic activity of glutathione S-transferase (GST), Generally the increase in the expression of GST is observed when the cell is stressed (**Di-Monte, 1992).**

In addition, GST is also involved in reducing the damage of ROS in different cells. It represents a family of multifunctional enzymes essentially cytosolic, involved in various operations of transport and intracellular biosynthesis **(Bidlack, 1996 ; Sauer, 2014 ; Di-Monte and Lavasani, 2002).**

1.3.5. Effect on CAT activity

To limit the excessive accumulation of ERO in the body, there are enzymatic and non-enzymatic defense systems, among the enzymatic systems: the CATwhich destroyH2O2 in H2O H2O **(Stanley and *al*., 2011; Lun and Holmgrn, 2013)**

The activity of catalase is specifically dependent on the transformation of hydrogen peroxide into oxygen and water **(Federico and *al*., 2012).** It is mainly located in the peroxisome. It therefore prevents oxidative damage within the peroxisome, and prevents its spread throughout the rest of the cell **(Fleming and *al.,* 1999).** Generally,

Imidacloprid alone or in admixture has caused a decrease in catalase activity in the brain. This observation suggests that these pesticides indirectly induce an increase of H2O2 in the mitochondria and the cytosol. Indeed, the decrease in GPx and CAT activities increases H2O2 and produces a massive amount of ° OH radical via the Fenton reaction **(Cory and *al.,* 2005 ; Banerjee, 2001).**

1.4. Effects of Imidacloprid on the activity of acetylcholine esterase

acetylcholine esterase (AChE) is a key enzyme found in the central nervous system and in the blood.

Neurotransmitters are among the neural molecules that can be affected by stress leading to neurobehavioral disturbance. Acetylcholine is the only neurotransmitter that acts on movement control that is not made from an amino acid. The regions of the brain with the highest density of neurons using choline are those that degenerate into Alzheimer's disease **(Nasuti and *al*., 2007 ; Songlin and *al*., 2015).**

The results of this study show a significant increase in the activity of AchE in the total brain in rats exposed to Imidacloprid.

This increase implies a decrease in Ach, reflecting a neurobehavioral variation such as a reduction in learning efficiency and the potential for memorization. These results are consistent with several results from other research evaluating the impact of pesticides on

neurobehavioral performance in the animal organism. **(Williams and Kauer, 1997 ; Nasuti and *al*., 2007 ; Pernot, 2009 ; Bhutada and *al*., 2011 ; Valerie and *al*., 2015).**

The use of meliss as a protective molecule against the deleterious effects of pesticides has significantly improved the redox status in the brain, which confirms its virtues against oxidative stress by indirectly strengthening cellular oxidizing systems or directly scavenging ROS.

Mitochondria are essential for the production of cellular energy necessary for the maintenance of cellular function ubcellular study **(Clayton and Doda, 2001 ; David and *al*., 2014 ; Lin and Beal, 2006).** Mitochondrial damage contributes to a decrease in ATP production **(Cassarino and Bennettjr, 1999; Datta and Kaviraj, 2003).** Alterations in one of the mitochondrial complexes cause the production of free radicals, which leads to depolarization of the mitochondrial membrane and a subsequent activation of voltage-dependent receptors, which allows the influx of calcium into the cell and the triggering of the pathways of cell death **(Lin and Beal, 2006; Romero and *al*., 2012; Rodriguez and *al*., 2016).** Indeed, the exposure of rats to Imidacloprid pesticides alone or in mixture has shown a significant increase in the mitochondrial permeability and swelling of the brain. This state of effects is the result of the action of ROS produced by the pesticides studied. It is well recognized that calcium overload is responsible for mitochondrial swelling which can cause the formation of nitric oxide (NO), and allow activating xanthine oxidase in the sense of generating the superoxide anion which inactivates NADH-dehydrogenase, NADH-oxidase and ATPase ultimately generating a state of mitochondrial oxidative stress **(Zhang and Darley, 2000).** The increase in mitochondrial swelling is due to the disruption of the giant pores dependent voltages under the effect of ROS causing massive entry of water and ions of Ca2 + and Na + via the mitochondrial membranes **(Romero and *al*., 2012 ; Morris and Berk, 2015; Henine and *al*., 2016; Taib and *al*., 2016).**

Cellular apoptosis is the end result of an attack by ROS, it is characterized morphologically by a dilation of the endoplasmic reticulum then, after an increase in the mitochondrial volume and swelling of the cell, there is a rupture of the membranes **(Ben-Haiand, 2014).**

1.5. Effects of imidacloprid and Melissa extract on the structural and functional integrity of the mitochondria

Mitochondria are essential for the production of cellular energy necessary for the maintenance of cellular function **(Clayton and Doda, 2001; David and *al*., 2014; Lin and**

Beal, 2006).Mitochondrial damage contributes to a decrease in ATP production **(Cassarino and Bennettjr, 1999; Datta and Kaviraj, 2003).** Alterations in one of the mitochondrial complexes cause the production of free radicals, which leads to depolarization of the mitochondrial membrane and subsequent activation of voltage-gated receptors, which allows the influx of calcium into the cell and triggering pathways of cell death **(Lin and Beal, 2006; Romero and *al*., 2012; Rodriguez and *al*., 2016**). Indeed, the exposure of rats to Imidacloprid pesticides alone or in mixture showed a significant increase in permeability and mitochondrial swelling in the brain. This state of effects is the result of the action of ROS produced by the pesticides studied. It is well recognized that calcium overload is responsible for mitochondrial swelling which can cause the formation of nitrogen monoxide (NO), and allow activation of xanthine oxidase in the sense of generating the superoxide anion which inactivates NADH-dehydrogenase, NADH-oxidase and ATPase ultimately generating a state of mitochondrial oxidative stress **(Zhang and Darley, 2000**). The increase in mitochondrial swelling is due to the disturbance of the voltages dependent giant pores under the effect of ROS causing the massive entry of water and Ca2 + and Na + ions via mitochondrial membranes **(Romero and *al*., 2012 ; Morris and Berk, 2015; Henine and *al*., 2016; Taib and *al*., 2016).** Cellular apoptosis is the final result of an attack by ROS, it is characterized morphologically by a dilation of the endoplasmic reticulum then, after increase in the mitochondrial volume and swelling of the cell, there is a rupture of the membranes **(Ben-Haiand , 2014).**

1.6. Effects of imidacloprid and Melissa extract on the neuro-behavior of rats

Numerous research reports have shown that exposure to stress has a complex effect on learning, locomotive activity, memory and mental development **(Cazakoff and *al*., 2010; Schwabe and *al*., 2012).** A behavioral disturbance was observed following the evaluation of the various parameters linked to neurobehavioural biology by applying the various tests in rats exposed to pesticides in order to evaluate their locomotor, emotional, exploratory, memorization and learning activity. The results of the classic labyrinth test (CL) and Vsoc-maze test (V) which showed behavioral change such as increased anxiety, fear, nervousness, short term memory loss, and disability thinking. These due to the gradual increase in mitochondrial ROS **(Shuichi and *al*., 2012).** This result is consistent with the previous results produced by several studies **(Griffith, 1991; Jùrgensen and Mouritsen, 2000; Kelley and *al*., 2011; Kim and *al*., 2005; Morris, 1984; Tsai and *al*., 2010)** . In this study, we found that the immobility time and the Exploration time of the imidiacloprid-treated rats changed compared to the control rats, demonstrating also a highly significant decrease in locomotor

activity of these rats. The labyrinth test is one of the most important behavioral models for assessing anxiety, memorization and learning. The increase in the number of tours, the time of arrival and the time spent in the midst of the cage are considered to be the most representative indicators of anxiolytic activity. The increase in arrival time at the end point in the classic labyrinth test in rats treated with the two insecticides alone or in mixture may be the result of a loss of tissue mass and therefore of neurodegeneration affecting the areas principles of memorization and learning by these pesticides **(Mani and *al*., 2014)**. This neurodegeneration causes a state of insufficient memory and of learning capacity in rats infected with these Neurotoxicity of imidiacloprid and the prevention of this toxicity by the extract Melissa in rats

Toxic. In this device, rats normally prefer to spend less of their time and less number of rounds. It should be noted that these results are consistent with the results of several previous **works (Walsh and Cumins, 1976; Belzing and Griebel, 2001; Bromley-Brits and *al*., 2011)**. Negative emotions such as anxiety, loss of learning, memory and depression cause an increased risk of disorders with an inflammatory or necrotic etiology, and increased inflammatory activity can be an important mediator of emotional relationships under intoxication by the pesticides studied **(Kahloula and *al*., 2014).**

Learning and memory are greatly affected by the stress generated by imidiacloprid. Acute or chronic stress experiences may be more sensitive to memory deficits than non-stressful situations. . Furthermore, the results of the test (V) showed that the mixture of pesticides causes a deficit in the reference memory in rats, revealed by the increase in latency time and a decrease in the number of entries and the time spent. in the targandquadrant. In the present study, using the sucrose test to further deepen and discover the psychological changes in rats after exposure to low doses of imidiacloprid. Analysis of the results of this test indicated a decrease in learning and memorization in these animals. This result is in agreement with recent studies on pesticide poisoning **(Guedri, 2014; Lahouel and *al*., 2016; Guedri and *al*., 2017**). On the other hand, the administration of the extract melisa at a dose of 150 µl / d for 20 days has greatly improved learning disabilities and memory retention. Recently, the role of the Melissa extract as a neuroprotective has been documented in several **studies (Gindin and *al*., 1995; Benhammou, 2011; Lahouel and *al*., 2016; Begoule and *al*., 2017).** Melissa extract increases acetylcholine production, supports mitochondria in energy production, prevents oxidative damage, increases brain oxygen consumption, promotes dopamine synthesis. In general, the virtues of this extract are magnificent, going as far as repairing damaged neurons **(Mona and *al.,* 2014; Gao and *al*., 2014)**

Conclusion and perspective

Conclusion and perspective

Pesticides, toxic chemicals, pose a real public health problem, both for users and for the population. Pesticides can also be very harmful, and they are suspected of posing a risk to human health and the environment by accumulating in ecosystems. Neonicotinoids are pesticides for agricultural use widely used by farmers. This present work presents a bibliographic study,Through this study, based on the evaluation of the oral toxicity of Imidacloprid at two doses (5 and 50 mg / kg / day) on body weight and nervous system function in Wistar rats, we have shown that the doses tested, especially the high and medium doses, have several harmful effects.

Im Imidacloprid tested and result in a significant reduction in physiological parameters such as body weight, weight gain and an increase in relative brain weights.

- * Imidacloprid tested and lead to a significant reduction in physiological parameters such as body weight, weight gain and an increase in relative brain weights.
- On the other hand, the biochemical parameter is also affected by the different treatments with a strong disturbance of the different cellular metabolites including proteins and lipids.
- The pesticide Imidacloprid have also caused alterations in the balance of oxidative stress which results in a disturbance in levels of GSH and MDA, the activity of CAT, GPx, GST.
- Regarding The effect of Imidacloprid on the mitochondria shows a toxic effect through the induction of mitochondrial permeability and swelling.
- The force-feeding of Melissa extract at a dose of 10 mg / kg / day for 20 days to rats treated with Imidacloprid restored all values to normal, which reflects the protective effect of the extract on the function of nervous systems.

In perspective, it would be interesting to develop this research from an operational point of view by deepening know ledge on:

-Investigation of the effect of this dose on the other vital organs

-extend the period of exposure of animals to force-feeding to this insecticide, in order to know whether the disturbances in the oxidant / antioxidant status observed could lead to the appearance of pathologies.

-Deepen the study with a histological study aimed at locating the tissue damage caused by pesticides.

References

References

1. a personal view J. Ethnopharmacol., 100 (1–2) (2005), pp. 131–134
2. **Abou-Donia MB, Goldstein LB, Bullman S, Tu T, Khan WA, Dechkovskaia AM, andAbdelRahman aA,(2008).** Imidacloprid induces neurobehavioral deficits and increases expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following in utero exposure. Journal of Toxicology and Environmental Health, Part A. p 119-130
3. Agarwal, R,2008. Severe neuropsychiatric manifestations and rhabdomyolysis in a patient with imidacloprid poisoning. *Am. J. Emerg. Med.* 2008, 25, 844-856.
4. **Ahmed Mai, Vogel CF, andMatsumura F,(2015).** Unique biochemical and molecular biological mechanism of synergistic actions of formamidine compounds on selected pyrethroid and neonicotinoid insecticides on the fourth instar larvae of Aedesaegypti (Diptera: Culicidae). Pesticide biochemistry and physiologie. P 057-63
5. **Aïna MP, Agbohessi P, Toko II, andScippo ML,(2015).** Effets toxicologiques andmethodes d'analyse de la lambda-cyhalothrine andde l'acetamipride utilises dans la protection phytosanitaire du cotonnier au Benin. International Journal of Biological and Chemical Sciences. p2184-2199.
6. ***Alain viala* and *alain* botta(2005),.**livre toxicolgie, 2eme edition .,acheve d'imrimer mes presses, Franve P988
7. Allen, T. R.; Frei, T.; Leutkemeier, H.; Vogel, O.; Biedermann, K.; Wilson, J. A 52-week oral toxicity (feeding) study with NTN 33893 technical in the dog. Unpublished Report no. R 4856, 1989, amendment no. R 4856A, 1992, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1989.
8. -Alsayeda, H.; Pascal-Lorber, S.; Nallanthigal, C.; Debrauwer, L.; Laurent, F. Transfer of the insecticide [^{14}C] imidacloprid from soil to tomato plants. *Environ. Chem.* Lett. 2008, 6, 229-234.
9. **Anadn A, Martinez L, Diaz M, Bringas P, Fernandez M (1991**) Effect of deltamethrin on antipyrine pharmaeokineties and metabolism in rat. Arch Toxicol 65: 156-159
10. -Andrew Chevallier.,2007. LAROUSSE des plantes medicinales.2nd Edition. 115 p
11. **ANSES,(2016),.** Index des RCP [en ligne]. Disponible sur : http://www.ircp.anmv.anses.fr/ [Consulte le 10 juin 2016]

12. **Antonio-Arreola GE, López-Bello R, Romero-Moreno DK, andSánchez D,(2011**). Laboratory and field evaluation of the effects of the neonicotinoid imidacloprid on the oviposition response of Aedes (Stegomyia) aegypti Linnaeus (Diptera: Culicidae). Memórias do Instituto Oswaldo Cruz. p997-100
13. **Aoun M, Tiranti V (2016)** Mitochondria: A crossroads for lipid metabolism defect in neurodegeneration with brain iron accumulation diseases. Inter Journal Bioch & Cell Bio 01-018
14. **Arora D, Haris S, Kumar S, Pratap S, Tripathi A, Mandal A, Shankar S, Kumar SH, Shukla H 2016** evaluation and physiological correlation of plasma proteomic fingerprints for Deltamethrin induced hepatotoxicity in Wistar rats. LFS 14866: 04-025
15. Avery, M,L., D. Decker, D.L. Fischer and T.R. Stafford. 1993. Responses of Captive Blackbirds to a New Seed Treatment. J. Wildl. Manage. 57(3): p652-656.
16. Avery, M,L., D.G. Decker and D.L. Fischer. 1994. Cage and Flight Pen Evaluation of Avian Repellancy and Hazard Associated with Imidacloprid-Treated Rice Seed. Crop Protection 13(7): 535-540.
17. **Baba Aissa (1999).** Ency clope die des plantes utiles : Flor d'Algerie anddu Maghreb. Ed : librarie Modern rouiba ; p243 – 244
18. **Banerjee A, Manna S, Saha SK,(2004).** Effect of aqueous extract of Azadirachta indica A. Juss (neem) leaf on oocyte maturation, oviposition, reproductive potentials and embryonic development of a freshwater fish ectoparasite Argulus bengalensis Ramakrishna, 1951 (Crustacea: Branchiura). Parasitol. Research. P 4641-4650
19. **Banerjee BD, Seth V, Ahmed RS (2001)** Pesticide-induced oxidative stress: perspectives and trends. Rev Environ Health 16: 1-40
20. Bartels A.,1986., Guide des plantes du bassin mediterraneen, Editions Eugen Ulm.er,
21. **Bear MF, Connors BW, Paradiso MA (2016).,** Neuroscience, Exploring the Brain. Fourth Edition. New York. ISBN 978-0-7817-7817-6. 612.8-dc23. 1019pp
22. Becker, H.; Vogel, W.; Terrier, C. Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rat. Unpublished Report no. R 4582, 1988, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1988a.
23. Becker, H.; Vogel, W.; Terrier, C. Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rabbit. Unpublished Report no. R 4583, 1988, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological*

Evaluations: Imidacloprid; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland,1988b.

24. **Beghoul A, Kebieche M, Gasmi S, Chouit Z, Amiour C, Lahouel A, Lakroun Z, Rouabhi R, Fetoui H, Soulimani R(2017)** Impairment of mitochondrial integrity and redox status in brain regions during a lowdose long-term exposition of rats to pyrethrinoïds: the preventive effect of quercetin. Environ Sci Pollut Res Doi : 10.1007/s11356-017-9675-0
25. **Belzing C and Griebel G (2001)** Measuring normal and pathological anxiety-like behavior in mice. Behavior Brain 141-149
26. **Belzunces, L. P., Tchamitchian, S. andBrunet, J-L. (2012)** Neural effects of insecticides in the honey bee. *Apidologie,* Vol. 43, n° 3, pp. 348-370. DOI 10.1007/s13592-012-0134-0.
27. **Benbouzib H (2012)** Evaluation andetude de la toxicite d'une famille d'acaricide sur des protistes cilies. Thèse de doctorat. Annaba University. 87pp
28. **Ben-Hai D and hoshan S (2014**) Assays of Mitochondrial Ca2+ Transport and Ca2+ Efflux via the MPTP. Cold Spring Harb Protocols. doi : 10.1101/073163S45-7
29. **Benhammou N (2011)** Activite antioxydante des extraits des composes phenoliques de dix plantes medicinales de l'Ouest anddu Sud-Ouest Algerien. Thèse doctorat. University of setif. 174pp
30. **Bernard G, Novel C and Vergnes R (2007).,** Neurologie andneurochirurgie. Estem, 260pp-
31. Bhardwaj S, Srivastava MK, Kapoor U, andSrivastava LP,2010. A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. Food and chemical toxicology . p1185-1190
32. **Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P, Umathe S, Mundhada D (2011**) Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain Res 30-41
33. **Bidlack WR (1996)** Interrelationships of food nutritiand health: the National Association of State Universities and Land Grant Colleges White Paper. Journal Amerc Coll Nutr 15: 422-33
34. **Block ML and Hong JS (2005).,** Microglia and inflammation mediated neurodegeneration: Multiple triggers with a commen mechanisme. Prog in neurobiology, p11-2

35. Bonda DJ, Bajic VP, Spremo-Potparevic B, Casadesus G, Zhu X, Smith M and Lee HG (2015)., Cell cycle aberrations and neurodegeneration. Neuropathology and Applied Neurobiology 36: 157-163

36. BONMATIN, J.-M., GIORIO, C., GIROLAMI, V. and *al.* (2015) Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research. Vol. 22, n° 1, pp. 35□6

37. Bradford (1976).a rapid and sensitive methodfor the quantitesof microgram quantities of protein utilising the principle of protein_dye binding.anal biochem72 :248_254

38. Braillon G (2002)., le système nerveux central a l'usage des etudiants en medecine. Ed: doin. 123pp-

39. Bromley-Brits K, Deng Y, Song W (2011) Morris water maze test for learning and memory deficits in Alzheimer's disease Model Mice. Journal Vis Exp 53: 29-20

40. Brooker C and Langlois WI (2001)., Lepresle E le corps humain : etude, structure andfonction : le rôle infirmier dans la pratique clinique, 2end edition, de boeck universite. 562pp

41. -Brunet, J.; Maresca, M.; Fantini, J.; Belzunces, L. P,2004. Human intestinal absorption of imidacloprid with Caco-2 cells as entercyte model. *Toxicol. Appl. Pharmacol.*2004, 194 (1), 1-9.

42. Buckingham M, Meilhac S and Zaffran S (2005)., Building the mammalian heart from two sources of myocardial cells. Nature Reviews Genetics 6: 826-836

43. Buckingham SD, Lapied B, Le-Corronc H, Grolleau F. and Sattelle DB,(1997). Imidaclopride actions on insect neuronal acetylcholine receptors .P200

44. Buckingham, S. D.; Lapied, B.1997,. Corronc, H. L.; Grolleau, F.; Sattelle, D. B. Imidacloprid Actions on Insect Neuronal Acetylcholine Receptors. *J. Exp. Biol.* 1997, 200, 2685-2692.

45. Cakmak I and Horst WJ (1991) Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max) Physiol. Plantarum. 83: 463-468

46. Calixto, (2005) Twenty-five years of research on medicinal plants in Latin America:

47. Calumpang, S. M. F.; Medina, M. J. B. Applicator Exposure to Imidacloprid while Spraying Mangoes. *Bull. Environ. Contam. Toxicol.*1996, 57, 697-704.

48. Cambier J, Masson M, Dehen H, Masson C (2008)., Neurologie. 12ème edition. Elsevier Masson. 540pp

49. Caramaschi D, de Boer S, Koolhaas JM (2007)., Differential role of the 5-HT 1A receptor in aggressive and non-aggressive mice. Physiology & Behavior. Doi : 10.1016/j.physbeh.2006.11.010

50. Carole I and Harve Q (2011) Desordres metaboliques andreanimation : de la physiopathologie au traitement. Berlin Heidelbeng. New York. ISBN: 978-2-287-99026-7. 522pp

51. CASIDA, J. E. andQUISTAD, G. B. (2004) Why insecticides are more toxic to insects than people: The unique toxicology of insects. Journal of Pesticide Science [en ligne]. Vol. 29, n° 2. Disponible sur : http://escholarship.org/uc/item/9299n2n7 [Consulte le 23 juin 2016]

52. Cassarino D, BennettJr J (1999) An evaluation of the role of mitochondria in neurodegenerative diseases: mitochondrial mutations and oxidative pathology. Protective nuclear responses and cell death in neurodegeneration. Brain Res Rev 29: 1-25

53. Cazakoff BN, Johnson KJ and Howland JG (2010) Con -verging effects of acute stress on spatial and recognition memory in rodents: a review of recent behavioral and pharmacological findings. Prog Neuro Psycho-pharmacol Biol Psychiatry 34: 733-741

54. Chakroun S, Ezzi L, Grissa I, Kerkeni E and *al.* (2016) Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environ Sci Pollut Res. Doi: 10.1007/s11356-016-9

55. Chakroun S, Ezzi L, Grissa I, Kerkeni E and *al.* (2016) Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environ Sci Pollut Res. Doi: 10.1007/s11356-016-9

56. Christensen HL, Nguyen AT, Pedersen FD, Damkier HH (2013)., Na+ dependent acid-base transporters in the choroid plexus; insights from slc4 and slc9 gene detetion studies. Front Physiol 4(304)

57. Clayton DA and Doda JN (2001) Isolation of mitochondria from cells and tissues. Press Beijing China 356-361

58. Cliona M and al. (2011) Strain differences in the neurochemical response to chronic restraint stress in the rat: relevance to depression. Pharmacol Biochem Behav 97: 690-699.

59. *control*47 (1), 77-81.

60. -Copping, L. G.,2001., *The Biopesticide Manual*, 2nd ed.; British Crop Protection Council: Surrey, England, 2001; pp 202-203.

61. **Cory-Slechta Da, Thiruchelvam M, Richfield Ek, Barlow Bk, Brooks Ai (2005) Developmental** pesticide exposures and the Parkinson's disease phenotype. Birth Defects Res A Clin Mol Teratol 73: 136-139
62. Costa, L. G.,2008., Toxic Effects of Pesticides. *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 7th ed.; Klaassen, C. D., Ed.; McGraw Hill: New York, NY, 2008; pp 907, 922-930.
63. -Cox, L.; Koskinen, W. C.; Yen, P. Y. Sorption-desorption of imidacloprid and its metabolites in soil. *J. Agric. Food Chem.* 1997, 45, 1468-1472.
64. **Crystal R, Saunders L, Salil K and *al.* (2006)** Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress Journal of Applied Toxicolog 26: 427-438
65. **Datta M and Kaviraj A (2003)** Acute Toxicity of the Synthetic Pyrethroid Deltamethrin to Freshwater Catfish Clarias gariepinus 296-299
66. **David A, Clayton S, Gerald S (2014**) Isolation of Mitochondria from Tissue Culture Cells, Cold Spring Harb Protocols. Doi : 10.1101/080002
67. **Debuigne Delphine andCouplan François**. Le petit Larousse des plantes qui guerissent. Editions Larousse, 2013. 1029p
68. Deepu, D.; George, I. A.; Peter, J. V,2007. Toxicology of the newer neonicotinoid insecticides: Imidacloprid poisoning in a human. *Clin. Toxicol.*2007, 45, 485-486.
69. Defebvre l and Verin M (2006) la maladie de parkinson. Elsevier Masson. 220pp-
70. **Desvergne B,(2006**). Transcriptional regulation of metabolism. Physiol. Rev.p465-514
71. **Di Monte D and Lavasani M (2002)** Manning-Bog Ab. Environmental factors in Parkinson's disease. Neurotoxicology 23: 487-502
72. **Di Monte D and Lavasani M (2002)** Manning-Bog Ab. Environmental factors in Parkinson's disease. Neurotoxicology 23: 487-502
73. **Di Monte D, Chan P, Sandy MS (1992)** Glutathione in Parkinson's disease: a link between oxidative stress and mitochondrial damage. Ann Neurol 32(1): 111-115
74. Dias BG and Ressler KJ (2014)., Parental olfactory experience influences behavior and neural structure in subsequent generations. Nature Neuroscienc 17(1): 89-99
75. Doull, J., C.D. Klassen, and M.O. Amdur (eds.). 1991. Cassarett and Doull's Toxicology. The Basic Science of Poisons. Fourth Edition. Pergamon Press, Elmsford, NY.

76. Draft list of initial pesticide active ingredients and pesticide inerts to be considered for screening under the Federal Food, Drug, and Cosmetic Act. *Fed. Regist.* June 18, 2007, 72 (116), 33486-33503.

77. dubois M, (1956) gilles Khamiltona JK and .al colorimetric method for determination of sugars .nature 1956 ;168(4265) :167

78. Duchatear and Flarkin

79. Dupont F., Guignard J.L., (2007). Botanique : systematique moleculaire. Edition Masson, 1ère edition, 285 p

80. Durand D., Damonb M., Gobertc M. 2013. Le stress oxydant chez les animaux de rente: principes generaux. Oxidative stress in farmanimals: General aspects. Cahiers de nutrition andde dietetique. 48. 218-224.

81. EFSA (European Food Security Autority),(2014), Reasoned opinion on themodification of the existing MRL for acetamiprid in bananas.EFSA Journal.P12

82. Eiben, R.; Rinke, M. NTN 33893,1989: Subchronic toxicity study on Wistar-rats (administration in the feed for 96 days). Unpublished Report no. 18187, 1989, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland,1989.

83. Elbert, A., Haas, M., Springer, B., Thielert, W., andNauen, R. (2008). Applied aspects of

84. Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholine esterase activity. Biochem Pharmacol 7(88)

85. Engel LS, Hill DA, Hoppin JA,(2005), Pesticide Use and Breast Cancer Risk among Farmers' Wives in the Agricultural Health Study. Is Epidemiologie. P121–135

86. Environmental Contamination and Toxicology 78, 349-352.

87. Esterbauer H, Gebicki J, Puhl H, Jungens G (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic Biol Med 13: 341

88. *Federal Register* October 12, 2005: Imidacloprid: Pesticide Tolerances for Emergency Exemptions., 70 (196), 59268-59276.

89. Federal Register,1995. Imidacloprid; Pesticide Tolerances. July 5, 1995. 60(128): 34943-24945.

90. Federico A and al. (2012) Mitochondria, oxidative stress and neurodegeneration. Journal Neurol Sci 05: 30

91. **Fleming L, Mann JB, Bean J, Briggle T, Sanchez-Ramos JR (1999**) Parkinson's diseaseand brain levels of organochlorinepesticides. Ann Neurol 4(36):100-103
92. **flohe and gunzler (1984)**;analysis of glutathion :peoxidase,methods enzymol.105 114-121
93. -Flores-Cespedes, F.; Gonzales-Pradas, E.; Fernandez-Perez, M.; Villafranca-Sanchez, M.; Socias-Viciana, M.; Urena-Amate, M. D. Effects of Dissolved Organic Carbon on Sorption and Mobility of Imidacloprid in Soil. *J. Environ. Qual.* 2002, 31, 880- 888.
94. Flucke, W. NTN 33893,1990: Tech.-study for subacute dermal toxicity in the rabbit. Unpublished report no. 19152, 1990, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Progamme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1990.
95. Fossen, M,2006., *Environmental Fate of Imidiacloprid*; California Department of Pesticide Regulation, Environmental Monitoring: Sacramento, CA, 2006.
96. **FOSSEN, M. (2006)** Environmental fate of imidacloprid [en ligne]. Environmental monitoring department of pesticide regulation. Disponible sur : http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/Imidclprdfate2.pdf [Consulte le 15 juin 2016]
97. **Ganong W and Jobin M (2005).,** physiologie medicale. 2end edition. de boeck university. 850pp
98. **Gao C, Chen X, Juan LJ, Tang Y, Liu L, Chen S, Yu H, Liu L, Yao P (2014**) Myocardial mitochondrial oxidative stress and dysfunction in intense exercise: regulatory effects of quercetin. Eur J Appl Physiol 114: 695-705
99. Gasmi Salim, Neurotoxicite de deux pesticides (Acetamipride andDeltamethrine) andla prevention de cette toxicite par la quercetine chez le rat, 2018, These Doctorat, Universite de Tebessa. 217p.
100. **Geoffroy AP, Etain P, Lajnef M and al. (2016**) Circadian genes and lithium response in bipolar disorders: associations with PPARGC1A (PGC-1) and RORA. Genes Brain and Behavior 15: 660-668
101. **Georges.B., Laval.D., Dr Lanzmann.P., Saïda.D.(2008).**Morphologie andphysiologie animales;Laboratoire de biologie, La dietetique de la longevite, Thèse de doctorat (specialite immunologie)
102. -Gerard D., 2003. L'encyclopedie des Plantes bio-indicateur. 3ème edition, Editions Promonature. 208 p.

103. Gerard T andGrabowski S (2002)., Principes d'anatomie andde physiologie, traduction : france boudreault, marie-claude desorcy, 3ème edition. de boeck university. 1256pp

104. GERVAIS, J. A., LUUKINEN, B., BUHL, K. (2010) Imidacloprid technical fact sheet. National Pesticide Information Center, Oregon State University

105. Gindin J, Novikov M, Kedar D (1995) The effect of plant phosphatidylserine on age-associated memory impairment and mood in the functioning elderly. Geriatric Institute for Education and Research and Dept of Geriatrics, Kaplan Hospital, Rehovot, Israel. 124pp

106. GIROLAMI, V., MAZZON, L., SQUARTINI, A. and al. (2009) Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. Journal of economic entomology. Vol. 102, n° 5, pp. 1808–181

107. GOULSON, D. (2013) An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology. Vol. 50, n° 4, pp. 977□987

108. Griffith CA (1991) possible case of instinctive behavior. Science 50: 166-167

109. Guedri K (2014) Etude des perturbations neuro-biologiques andcomportementales chez un modèle animal, gonadectomise. These doctorat. Annaba. 87pp

110. Guedri K, Frih H, Chettoum A, Rouabhi R (2017) Chronic Restraint Stress Induced: Neurobehavioral Alterations and Histological Changes in Rat. ToxEHS Doi: 10.1007/s13530-017-0312-6

111. Gueguen B, Chauvel P, Touchon J (2005)., Neurophysiologie des memoires. Elsevier Masson. 240pp

112. **Gupta, S., andGajbhiye, V.T.** (2007). Persistence of acetamiprid in soil. *Bulletin of*

113. -Gupta, S.; Gajbhiye, V. T.; Agnihotri, N. P. Leaching Behavior of Imidacloprid Formulations in Soil. *Bull. Environ. Contam. Toxicol.*2002, 68, 502-508.

114. Habig and al (1974)WH ,pabst MJ .,jakoby WB 1974 glutathion-s-transferase the first step in meracapturicacid formation .journal of biology and chimestry .249,7130-9

115. Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now?. Journal of Neurochemistry 97(6): 1634–1658

116. Hammoudi R., (2015). Activites biologiques de quelques metabolites secondaires extraits de quelques plantes medicinales du Sahara meridional algerien. Thèse de doctorat ès sciences, biologie, Universite Kasdi Merbah – Ouargla, 5 ; 23 ; 62 ; 63p

117. Hatcher J, Kurt D, Pennelland G, Miller W (2008) Parkinson's disease and pesticides: atoxicological perspective. Trends in Pharmacological Sciences 29(6)

118. Hatcher, J.M., Pennell, K.D., andMiller, G.W. (2008). Parkinson's disease and pesticides: a toxicological perspective. *Trends in Pharmacology Sciences* 29, 322-329.

119. *Hazardous Substances Databank (HSDB), Imidacloprid*,2006. U.S. Department of Health and Human Services, National Institutes of Health, National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~rEOnhW:1 (accessed October 2008), updated April 2006.

120. He Y, Zaho J, Zheng Y, Deesneux, We K,(2012) . Lethal effect of imidaclopride on the coccinellid predator Serangium japonicum and sublethal effects on predator voracity and on functional response to the whitefly tabaci . Ecotox.P 21

121. Hellpointer, E. 1994. Degradation and Translocation of Imidacloprid (NTN 33893) Under Field Conditions on a Lysimeter. Miles Report No. 106426, pp. 1-71. Miles Inc., Agricultural Division, PO Box 4913, Kansas City, MO.

122. Henine S, Rouabhi R, Gasmi S, Amrouche A, Abide A, Salmi A, Toualbia N, Taib C, Bouteraa Z, Chenikher H, Boussekine S, Kebieche M, Aouimeur M (2016) Oxidative stress status, caspase-3, stromal enzymes and mitochondrial respiration and swelling of Paramecium caudatum in responding to the toxicity of Fe3O4 nanoparticles. Environ Health Sci 8(2): 161-167

123. Hiltenbrand V. (1999). Place de la peroxydation lipidique dans les effets deletères des UV-A: mise en evidence des haptènes amino-imino-propène: interêts potentiels des antioxydants lipophiles en cosmetologie. thèse pour l'obtention de diplôme de Doctorat en pharmacie. Universite de JOSEPH FOURIER. 122p.

124. HOVDA, L. R. andHOOSER, S. B. (2002) Toxicology of newer pesticides for use in dogs and cats. Veterinary Clinics of North America: Small Animal Practice. Vol. 32, n° 2, pp. 455–467

125. -Hovda, L. R.; Hooser, S. B,.2002. Toxicology of newer pesticides for use in dogs and cats. *Vet. Clin. Small Anim.* 2002, 32, 455-467.

126. Hoyer D, Hannon JP, Martin GR (2002)., Molecular, pharmacological and functional diversity of 5-HT receptors. Pharma Biochem and Behavior 71: 533-554

127. Huang, N.; Lin, S.; Chou, C.; Hung, Y.; Chung, H.; Huang, S,2006. Fatal ventricular fibrillation in a patient with acute imidacloprid poisoning. *Am. J. Emerg. Med.* 2006, 24 (7), p883-885.

128. Imbert M (2006)., Traite du cerveau. Odile Jacob. 532pp

129. IWASA, T., MOTOYAMA, N., AMBROSE, J. T. and *al.* (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera. Crop Protection. Vol. 23, n° 5, pp. 371□378

130. Jenkins, J.J. 1994. Use of Imidacloprid for Aphid Control on Apples in Oregon. Potential for Ground and Surface Water Contamination. Department of Agricultural Chemistry. Oregon State University, Corvallis, OR.

131. JESCHKE, P. andNAUEN, R. (2008) Neonicotinoids-from zero to hero in insecticide chemistry. Pest Management Science. Vol. 64, n° 11, pp. 1084□1098

132. Jeschke, P. andNauen, R. (2008). Neonicotinoids-from zero to hero in insecticide chemistry.

133. JESCHKE, P., NAUEN, R., SCHINDLER, M. and *al.* (2011) Overview of the Status and Global Strategy for Neonicotinoids. Journal of Agricultural and Food Chemistry. Vol. 59, n° 7, pp. 2897□2908

134. Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global

135. Johnson, Reed M, Ellis, Marion D, Mullin, Christopher A,(2009) . Pesticides and honey bee toxicity–USA. Apidologie.p 312-331

136. Jùrgensen K and Mouritsen M (2000) Effects of the insecticides malathion and deltamethrin on the phase behaviour of dimyristoylphosphatidylcholine multilamellar lipid bilayers 1-8

137. Kahloula K, Houari D, Slimani M, Terras H, Achour S (2014) Effandde l'exposition chronique au nickel sur les fonctions neurocomportementales chez les rats Wistar pendant la periode de developpement. Toxicologie Analytique & Clinique 186-192

138. Kazushige M and *al.* (2000)., Chronic Stress Induces Impairment of Spatial Working Memory Because of Prefrontal Dopaminergic Dysfunction. Journal Neurosci 20: 1568-1574

139. Kelley B, Yu D, Weihong S (2011) Morris Water Maze Test for Learning and Memory Deficits in Alzheimer's disease. Columbia University. Dio: 10. 3791/2920-23-Z4345

140. Kelley B, Yu D, Weihong S (2011)., Morris Water Maze Test for Learning and Memory Deficits in Alzheimer's disease. Columbia University. Dio: 10. 3791/2920-23-Z4345

141. Kim JH, Kim M, Choi K, Son J (2016) Quercetin sensitizes pancreatic cancer cells to TRAIL-induced apoptosis through JNK-mediated cFLIP turnover. The

International Journal of Biochemistry & Cell Biology doi:10.1016/j.biocel.2016.07.033

142. Klein, O.; Karl, W.1990. Methylene [^{14}C] imidacloprid: metabolism part of the general metabolism study in the rat. Unpublished Report no. PF 3316, 1990, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; World Health Organization, International Programme on Chemical Safety: 1990.

143. Klein, O.1987,. [^{14}C]-NTN 33893: Biokinetic part of the 'general metabolism study' in the rat. Unpublished Report no. PF2889, 1987, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1987a.

144. Koistinaho M, Lin S, Wu X (2004)., Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-beta peptides. Nature Medcine 10: p719-726

145. Kolb B and Whishaw IQ (2002)., Cerveau andcomportement. de boeck university. 672pp

146. Kummer MP, Hermes M, Delekarte A (2011)., Nitration of tyrosine 10 critically enhances amyloid beta aggregation and pla-que formation. Neuron 71: 833-844

147. *Label Review Manual*; U.S Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs. http://www.epa.gov/oppfead1/labeling/lrm/chap-07.pdf (accessed June 2009), updated Aug 2007.

148. Lacombe M (2006)., abrege d'anatomie andde physiologie humaine. 6ème edition. de boock. 229pp

149. Lacombe M (2006)., abrege d'anatomie andde physiologie humaine. 6ème edition. de boock. 229pp-

150. Lahouel A, Kebieche M, Lakroun Z, Rouabhi R, Fetoui H, Chtourou Y, Zama D, Soulimani R (2016) Nurobehavioral deficits and brain oxidative stress induced by chronic low dose exposure of persistent organic pollutants mixture in adult female rat. Environmental Science and Pollution Research. Doi: 10.1007/s11356-016-6913-9

151. Lahouel A, Kebieche M, Lakroun Z, Rouabhi R, Fetoui H, Chtourou Y, Zama D, Soulimani R (2016) Nurobehavioral deficits and brain oxidative stress induced by

chronic low dose exposure of persistent organic pollutants mixture in adult female rat. Environmental Science and Pollution Research. Doi: 10.1007/s11356-016-6913-9

152. Lauvverys R, Vincent H, Dominique L (2007) Toxicologie industrielle andintoxication professionnelles. Masson 31-288pp

153. Lin MT and Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nat 787-95

154. -Liu, W.; Zheng, W.; Ma, Y.; Liu, K,2006. Sorption and Degradation of Imidacloprid in Soil and Water. *J. Environ. Sci. Health B* 2006, 41, 623-634.

155. Lodish H, Berk A, Matsudaira P, Darnell J, Kaiser CA, Masson PL (2005)., biologie moleculaire de la cellule. Masson. 3ème edition. de-boeck university. 1096pp

156. Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, andTelang A,(2014). Evaluation of imidacloprid-induced neurotoxicity in male rats: A protective effect of curcumin. Neurochemistry international.p 122-129

157. Lukaszewicz-Hussain A (2008) Subchronic intoxication with chlorfenvinphos, an organophosphate insecticide, affects rat brain antioxidative enzymes and glutathione level. Food Chem Toxicol 46(1): 8296

158. Maiza A., Kilani-Morakchi S., Rehamnia F., Bensbaa F. andAribi N(2006). Activie d'un Biopesticide. Le Spinosad. Chez Blatiella Germanica: Effets Sur Divers Biomarqueurs (LDH, GSH, MDA). Bull. Soc. zool. Fr. 136(1-4): 189-204

159. Mani MV, Asha S, Sadiq MMA (2014) Pyrethroid deltamethrin-induced developmental neurodegenerative cerebral injury and ameliorating effect of dietary glycoside naringin in male wistar rats. Biomed Aging Pathol 4: 1-9

160. Marieb E (2008)., Biologie humaine. Principes d'anatomie andde physiologie. Huitième edition. Canada : pearson education. 631pp

161. Martin C, Riou B, VallandB (2006)., physiologie humaine appliquee. Editions Arnette 1098pp

162. Martínez-Torres, S., Gomis-González, M., Navarro-Romero, A., Maldonado, R. and Ozaita, A. (2019). Use of the Vsoc-maze to Study Sociability and Preference for Social Novelty in Rodents. *Bio-protocol* 9(20)

163. Martoga R. andMartoga-Pierson M.(1967). «Initiation aux Techniques de l'Histologie animale», Paris: Masson.p345

164. MATSUDA, K., BUCKINGHAM, S. D., KLEIER, D. and *al.* (2001) Neonicotinoids : insecticides acting on insect nicotinic acetylcholine receptors. Trends in Pharmacological Sciences. Vol. 22, n° 11, pp. 573□580

165. Matsuda, K., Buckingham, S. D., Klleier, D. and *al*. (2001) Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences*. Vol. 22, n° 11, pp. 573-580. DOI 10.1016/S0165-6147(00)01820-4.

166. Matsuda, K.; Buckingham, S. D.; Freeman, J. C.; Squire, M. D.; Baylis, H. A.; Sattelle, D. B,.1998. Effects of the alpha subunit on imidacloprid sensitivity of recombinant nicotinic acetylcholine receptors. *Br. J. Pharmacol.*1998, 123, 518-524.

167. Matsuda, K.; Sattelle, D. B,.2005. Mechanism of Selective Actions of Neonicotinoids on Insect Acetylcholine Receptors. *New Discoveries in Agrochemicals: American Chemical Society Symposium Series*; Clark, J. M.; Ohkawa, H., III, Eds.; Oxford University Press: Oxford, UK, 2005; pp 172-183.

168. Meiser J, Weindl D, Hiller K, Meiser and *al*. (2013)., Complexity of dopamine metabolism. Cell Communication and Signaling, 11:34

i. **Meister, R.T. (ed.). (1995).** Farm Chemicals Handbook '95. Meister Publishing Company. Willoughby, oH

169. Meneses A and Perez-Garcia G (2007)., 5-HT1A receptors and memory. Neuroscience and Biobehavioral Reviews 31: 705-727

170. Michael F and Hughes EA (2016) Environmentally relevant pyrethroid mixtures: A study on the correlation of blood and brain concentrations of a mixture of pyrethroid insecticides to motor activity in the rat. Toxicology 359-360(10): 19-29

171. Mona K, Abdel A, Hanan A, Marwa A (2014) Vitamin E attenuates neurotoxicity induced by deltamethrin in rats. BMC CAlM. 1(1): 44-58

172. Mondal S, Ghosh RC, Mate MS, andKarmakar DB,(2009). Effects of acetamipride on immune system in female wistar rat. Proceedings of zoological society. p109-117

173. Morel Jean-Michel. Traite pratique de phytotherapie. Editions Grancher,

174. Morris G and Berk M (2015) The many roads to mitochondria dysfunction in neuroimmune and neuropsychiatric disorders. BMC medicine 13: 68

175. mortality of coccinellid larvae related to zoophytophagy. *Biological control* 51 (3), 487-492.

176. Moser, S.E., and Obrycki, J.J. (2009). Non-targandeffects of neonicotinoid seed treatments;

177. Moullan M, Ahossi V, andZwetyenga N,(2016). Le syndrome de Stevens-Johnson–toxicepidermalne crolysis (SJSTEN) lie à un insecticide: second cas dans la litterature

andimplications potentielles. Revue de Stomatologie, de Chirurgie Maxillo-faciale andde Chirurgie Orale. p176-182

178. Multigner L. Effets retardes des pesticides sur la sante humaine. Environnement, risques & sante, p187-194

179. Nabiuni M, Parivar K, Noorinejad R, Falahati Z, Khalili F, andKarimzadeh L,(2015). The reproductive side effects of Imidacloprid in pregnant Wistar rat. International Journal of Cellular & Molecular Biotechnology. p10-18

180. Nasuti C, Gabbianelli R, Falcioni M, Stefano A, Sozio P, Cantalamessa F (2007) Dopaminergic system modulation behavioral changes and oxidative stress after neonatal administration of pyrethroids. Toxicology 229: 194-205

181. Nasuti C, Gabbianelli R, Falcioni M, Stefano A, Sozio P, Cantalamessa F (2007) Dopaminergic system modulation behavioral changes and oxidative stress after neonatal administration of pyrethroids. Toxicology 229: 194-205

182. Nauen R,(2006) . Insecticide mode d'action of action : retum of the ryanodine receptor. Pest Man Sci.P 62

183. NAUEN, R., JESCHKE, P. andCOPPING, L. (2008) In focus: neonicotinoid insecticides editorial. Pest Management Science. Vol. 64, n° 11, pp. 1081□1081

184. neonicotinoid uses in crop protection. *Pest Management Science* 64, 1099-1105.

185. Newman, D.J. , Cragg, G.M. andSnadern, K.M.(2000) The influence of natural

186. Nicolle-Mir L,(2011). Perchlorate dans l'eau de boisson andfonction thyroïdienne des nouveau-nes. Environnement risques andsante.,p173-174.

187. Nieuwenhuys R, Voogd J, Huijzen CV (2008)., The human central nervous system. 4ème edition illustree. Springer. 967pp

188. Oi, M.,1999,. Time-Dependent Sorption of Imidacloprid in Two Different Soils. *J. Ag. Food Chem.* 1999, 47, 327-332.

189. Oliver, D. P.; Kookana, R. S.,2005., Quintana, B. Sorption of Pesticides in Tropical and Temperate Soils from Australia and the Philippines. *J. Ag. Food Chem.* 2005, 53, 6420-6425.

190. Papachristos, D.P., andMilonas, P.G. (2008). Adverse effects of soil applied insecticides on

191. Pascal.H (2010)., Le systeme nerveux. 2ème eddition. 44-62pp

192. Pauluhn, J. NTN 33893,1988: Subacute inhalation toxicity study on the rat according to OECD Guideline No. 412. Unpublished Report no. 18199, 1988, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations:*

Imidacloprid; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1988.

193. Pernot F (2009) Evenements moleculaires andcellulaires associes à l'epileptogenèse dans deux modèles murins d'injection intrahippocampique de toxiques. Implication des mecanismes neuro-inflammatoires, These doctorat, universite Jouseph fourier. 239pp

194. Pest Management Science 64, 1084-1098.

195. -Peterson BS and al. (2015) Effects of prenatal exposure to air pollutants (polycyclic aromatic hydrocarbons) on the development of brain white matter, cognition, and behavior in later childhood. Jama psychiatry 72(6): 531-540

196. Pihlaja R, Koistinaho J, Kauppinen R, Sandholm J, Tanila H, Koistinaho M (2011)., Multiple cellular and molecular mechanisms are involved in human Abeta clearance by transplanted adult as-trocytes. Glia 59: 1643-1657

197. Pike, K.S., G.L. Reed, G.T. Graf and D. Allison. 1993. Compatibility of Imidacloprid with Fungicides as a Seed-Treatment Control of Russian Wheat Aphid (Homoptera: Aphidae) and Effect on Germination, Growth, and Yield of Wheat Barley. J.Econ.Entomol. 86(2): 586-593.

198. products upon drug discovery. Natural Product Report. P :215-234.

199. Purves D, Augustine G, Fitzpatrick D, Coquery JM (2004)., Neurosciences. 2ème Ed. de boeck university. 752pp

200. Quezel P., Santa S., 1962/1963 –Nouvelle de l'Algerie anddes regions desertiques meridionales. Vol. l -2. Paris, C,N,R,S .l l70p

201. Raghavan, M.; Knapp, D. W.; Dawson, M. H.; Bonney, P. L.; Glickman, L. T,2004. Topical flea and tick pesticides and the risk of transitional cell carcinoma of the urinary bladder in Scottish terriers. *J. Am. Vet. Med. Assoc.* 2004, 225 (3), 389-394.

202. Raymond-Delpech V, Matsuda K, Sattelle BM, Rauh JJ, etSattelle DB,(2005). Ion channels: molecular targets of neuroactive insecticides. Invertebrate Neuroscience. p119-133

203. Reynolds P, Von Behran J, Gunier RB , Goldberg DE, Hertz A, Harnly M,(2002). Childhom cancer and agricultural pesticide use: an ecologic study in california. Environ Health prespect. p319-329.

204. Riedel D, Tremblay N, Tompkins E,(1997). Stade of Knowledge Report on Environmental Contaminants and Human Health in the Creat Lakes Basin. Sante Canada: Great Lakes Program Environmental Effects Division.

205. Rjeibi I (2016) Oxidative damage and hepatotoxicity associated with deltamethrin in rats: The protective effects of Amaranthus spinosus seed extract. Biomedicine & Pharmacotherapy 84(8): 853-860

206. Roberts, T. R., Hutson, D. H,1999. Imidacloprid. *Metabolic Pathways of Agrochemicals - Part 2: Insecticides and Fungicides*; The Royal Society of Chemistry: Cambridge, UK, 1999; pp 111-120.

207. Robitaille CN,(2014). Effets antiandrogeniques de pesticides dans les cellules cancereuses corticosurrenales humaines andde la prostate humaine (Doctoral dissertation, Universite du Quebec, Institut National de la Recherche Scientifique). P130

208. Rodrigues KJA, Santana MB, Do Nascimento JLM, Picanco-Diniz DLW, Maues, LAL, Santos SN, ... andFaro, L.R.F,2010. Behavioral and biochemical effects of neonicotinoid thiamethoxam on the cholinergic system in rats. Ecotoxicology and environmental safety, p101-107

209. Rodriguez ME, Martinez F, Espinosa M, Maldonado S (2016) Mitochondrial Dysfunction in the Hippocampus of Rats Caused by Chronic Oxidative Stress. Neuroscience 08-018

210. Romero A, Ramos E, Castellano V, Martínez MA, Ares I, Martínez M, Martínez-Larrañaga MR, anadón A **(2012)** Cytotoxicity induced by deltamethrin and its metabolites in SH-SY5Y cells can be differentially prevented by selected antioxidants. Toxicol In Vitro 26: 823-830

211. Rouabhi and al (2006;2009) toxic effects of combined melecul from novaluron and diflubenzuron on paramecium .am-Euras J toxicol sci 2009 ;1(1) :74-80

212. Rouabhi R, Gasmi S, Boussekine S, Kebieche M (2015) Hepatic oxidative stress induced by zinc and opposite effect of selenium in oryctolagus cuniculus. Journal Environ Anal Toxicol 5: 289-298

213. Rouabhi R, Gasmi S, Boussekine S, Kebieche M (2015) Hepatic oxidative stress induced by zinc and opposite effect of selenium in oryctolagus cuniculus. Journal Environ Anal Toxicol 5: 289-298

214. Rouchard, J., F. Gustin and A. Wauters. 1994. Soil Organic Matter Aging and its Effect on Insecticide Imidacloprid Soil Biodegradation in Sugar BeandCrop. Toxicol. Environ. Chem. 45(3-4): 149-155.

215. -Rouchaud, J.; Gustin, F.; Wauters, A. Imidacloprid Insecticide Soil Metabolism in Sugar BeandField Crops. *Bull. Environ. Contam. Toxicol.*1996, 56, 29-36.

216. -Rouchaud, J.; Gustin, F.; Wauters, A. Soil Biodegradation and Leaf Transfer of Insecticide Imidacloprid Applied in Seed Dressing in Sugar BeandCrops. *Bull. Environ. Contam. Toxicol.*1994, 53, 344-350.

217. Rustin P, Chretien D, Bourgeron T, Gerard B, Rotig A, Munnich A (1994) Biochemical & mol investigation in respiratory chain deficiences. Clin Chem Acta 35-51

218. Samuel O, Dion S, St-Laurent L,(2012). Indicateur de risque de pesticides du Quebec: IRPeQ: sante andenvironnement (2e edition). Ministère de l'Agriculture, des Pêcheries andde l'Alimentation du Quebec.

219. SÁNCHEZ-BAYO, F. andHYNE, R. V. (2014) Detection and analysis of neonicotinoids in river waters – Development of a passive sampler for three commonly used insecticides. Chemosphere. Vol. 99, pp. 143□151

220. Sarkar, M. A.; Roy, S.; Chowdhury, A. Persistence and metabolism of imidacloprid in different soils of west Bengal. Pest Manag. Sci. 2001, 57, 598-602.

221. Sauer E (2014) Liver delta aminolevulinate deshydratase activity is inhibited by neonicotinoids and restored by antioxidants agents Int J Environ Res Public Health 11(11): 11676-11690

222. Scholz, K., and M. Spiteller. 1992. Influence of Groundcover on the Degradation of 14C-Imidacloprid in Soil. Brighton Crop Protection Conference. Pests and Diseases. pp. 883-888.

223. Schroeder, M. E.; Flattum, R. F,1984. The Mode of Action and Neurotoxic Properties of the Nitroethylene Heterocycle Insecticides. *Pestic. Biochem. Physiol.* 1984, 22, 148-160.

224. -Schulz-Jander, D. A.; Casida, J. E,.2002. Imidacloprid insecticide metabolism: human cytochrome P450 isozymes differ in selectivity for imidazolidine oxidation versus nitroimine reduction. *Toxicol. Lett.*2002, 132 (1), 65-70.

225. Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS (2012) Stress effects on memory. Neurosci Biobehav Rev 36: 1740-1749

226. *Sci*. 28, 14–22.

227. Shadnia, S,2007. Fatal intoxication with imidacloprid insecticide. *Am. J. Emerg. Med.* 2007, 26, 634.e1-634.e4.

228. Shalaby SE, Farrag ARH, Farrag ARH, andGamila SM. Toxicological potential of thiamethoxam insecticide on Albino rats and its residues in some organs. JASMR 2010; p 165-172

229. Sheehan D., Meade G., Foley V.-M. andDowd C.A. (2001). Structure, function and evolution of glutathione transferases : implications for classification of non mammalian members of an ancient enzyme superfamily . Biochem. J. 360, 1-16

230. -Sheets, L. P,2001. Imidacloprid: A Neonicotinid Insecticide. *Handbook of Pesticide Toxicology*, 2nd ed.; Krieger, R. I., Ed.; Academic Press: San Diego, CA, 2001; Vol. 2, Chapter 54, pp 1123-1130.

231. Sheets, L. P,2002. The Neonicotinoid Insecticides. *Handbook of Neurotoxicology*; Massaro, E. J., Ed.; Humana Press: Totowa, NJ, 2002; Vol. 1, Chapter 6, pp 79-87.

232. Shuichi C and *al.* (2012)Chronic restraint stress causes anxiety-and depression-like behaviors, down regulates gluco -corticoid receptor expression and attenuates glutamate release induced by brain derived neurotrophic factor in the prefrontal cortex. Prog Neuro Psychopharmacol Biol Psychiatry 39: 112-119

233. Siegel A and Sapru N (2005)., Essential neuroscience, edition illustree, lippincott williams & wilkins. 576pp

234. SIMON-DELSO, N., AMARAL-ROGERS, V., BELZUNCES, L. P. and al. (2015) Systemic insecticides (neonicotinoids and fipronil) : trends, uses, mode of action and metabolites. Environmental Science and Pollution Research. Vol. 22, n° 1, pp. 5□34

235. Site web (provence arome.fr)

236. Songlin C, Xiaoli Y, Yinying L, Weiyi M, Xiaoyun L, Changran Z (2015) Alzheimer's disease treated with combined therapy based on nour-ishing marrow and reinforcing Qi. Journal Tradit Chin Med June 255: 25

237. Specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology* 48,

238. Stanley B.A., Sivakumaran V., Shi S., Mcdonald I., Lioyd D., Watson W.H.,Aon M.A. andPaocci N. (2011). Thioredoxin reductase-2 is essnetial for keeping low levels of H2O2emission from isolated heart mitochondria; J.Biol.Chem.; 286, 33669-33677

239. Strategy for neonicotinoids. *J. Agric. Food Chem.* 59, 2897–2908.

240. Suchail S, Guez D, Belzunces LP,(2003). Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in Apismellifera. Environ Toxicol Chem,.p6

241. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH,(2004). Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. p228-238

242. Suter, P.; Vogel, W.; Wilson, T.; Terrier, C. Multiple-generation reproduction study with NTN 33893 technical in rats. Unpublished Report no. R5097, 1990, amendment report no. R5097A, 1990, and amendment report no. R5097B, 1992, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1990.

243. Taib C, Rouabhi R, Gasmi S, Bakhouche L, Zarai G, Toualbia N, Salmi A, Henine S, Chenikher H, boussekine S, Kebieche M, Aouimeur M, Djabri B (2016) Toxicity of Fe3O4 Nanoparticles. ToxEHS 8(5): 349-355

244. Tebourbi O, Hallègue D, Yacoubi MT, Sakly M, andRhouma KB,(2010). Subacute toxicity of p, p′DDT on rat thyroid: Hormonal and histopathological changes. Environmental toxicology and pharmacology.,p 271-279

245. Thany, S.H., Lenaers, G., Raymond-Delpech,V., Sattelle, D.B., Lapied, B., 2007. Exploring

246. The pharmacological properties of insect nicotinic acetylcholine receptors. *Trends Pharmacol.*

247. the predatory coccinellid Hippodamia undecimnotata (Coleoptera: Coccinellidae) *Biological*

248. Thyssen, J.; Machemer, L,1999. Imidacloprid: Toxicology and Metabolism. *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*; Yamamoto, I.; Casida, J. E., Eds.; Springer-Verlag: Tokyo, 1999; Chapter 9, pp 213-222.

249. Tomizawa, M., Casida, J. E.,1999. Minor structural changes in nicotinic insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors. *Br. J. Pharmacol.*1999, 127 (1), 115-122.

250. Tomizawa, M., andCasida, J.E. (2003). Selective toxicity of neonicotinoids attributable to

251. Tomizawa, M.; Casida, J. E.,2005. Neonicotinoid Insecticide Toxicology: Mechanisms of Selective Action. *Annu. Rev. Pharmacol. Toxicol.*2005, 45, 247-268.

252. Tomlin, C. D. S,.2006,. *The Pesticide Manual, A World Compendium*, 14th ed.; British Crop Protection Council: Surry, England, 2006; pp 598-599.

253. Tomlin,.2006. Novel Insecticides. *Clinical Veterinary Toxicology*; Plumlee, K. H., Ed.; Mosby: St. Louis, MO, 2004; pp 184-185.

254. Toumi H (2013) Ecotoxicite de la deltamethrine anddu malathion sur differentes souches de Daphnia magna. these doctrtat. 208p

255. Tritsch d, chesnoy-marchais d, feltz a, physiologie du neurone, 2ème Ed, editions doin, 1999, 715 p-

256. Tsai F, Cheng H, Hsieh M, Wu C, Lin Y, Peng W (2010) The ameliorating effects of luteolin on betaamyloid-induced impairment of water maze performance and passive avoidance in rats. Am Journal Chin Med 279-91

257. U.S. Environmental Protection Agency,. 1995. Imidacloprid; Pesticide Tolerance and Raw Agricultural commodities. 40 CFR Part 180 Section 472.

258. U.S. Environmental Protection Agency. 1995. [^{14}C]-NTN 33893,1987: Investigations on the distribution of the total radioactivity in the rat by whole body autoradiography. Unpublished report no. PF2891, 1987, submitted to WHO by Bayer AG, Mannheim, Germany. *INCHEM Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization: Geneva, Switzerland, 1987b.

i. -U.S. Environmental Protection Agency. 1995. Imidacloprid; Pesticide Tolerance and Raw Agricultural commodities. 40 CFR Part 180 Section 472.

259. Valerie P, Sylvain M, Virginie G, Edwin P, Mathieu D, Patrick K (2015) Effects of low dose endosulfan exposure on brain neurotransmitter levels in the African clawed frog Xenopus laevis. Chemosphere 357364

260. Verkhratsky A and Butt A (2007)., Glial neurobiology : a textbook, edition : illustree, editeur : john wiley and sons. 215pp

261. Vidal, Editions Vidal, Fevrier 2018. 3932p

262. Viviana VL, Angelica TB, Lina GM, Alejandro M, Marisol RL (2015) Acute restraint stress and corticosterone transiently disrupts novelty preference in an object recognition task. Behav Brain Res 291: 60-66

263. Walsh N and Cumins A (1976) The OF-test a critical. Psychol Bull 83: 482-504

264. Wang XF, Li S, Chou AP, Bronstein JM (2006)., Inhibitory effects of pesticides on proteasome activity: implication in Parkinson's disease. Neurobiol Dis 23(1): 198-205

265. Ware, G. W.; Whitacre, D. M,.2004. *The Pesticide Book*; MeisterPro Information Resources: Willoughby, OH, 2004; pp 70-71.

266. weckbeker and Cory glutathione (1988). 1988.ribonucleotide reductase activity and growth of glutathione depended mouse leukemia L1210celss in vitro .cancer letter ,40,257-264.PMID :3289734

267. -Westwood, F.; Bean, K. M.; Dewar, A. M.; Bromilow, R. H.; Chamberlain, K. Movement and Persistence of [^{14}C] Imidacloprid in Sugar-BeandPlants Following Application to Pelleted Sugar-BeandSeed. *Pestic. Sci.* 1998, 52, 97-103.

268. WHO. *Toxicological Evaluations: Imidacloprid*; International Programme on Chemical Safety, World Health Organization. http://www.inchem.org/jmpr/jmprmono/2001pr07.htm (accessed Oct 2008), updated Feb 2004.

269. William.P (1981)., Anatomie andphysiologie in organisation generale andphysiologie humain. p209-214

270. Williams JH and Kauer JA (1997) Properties of carbachol-induced oscillatory activity in rat hippocampus. Journal of Neurophysiology 2631-2640

271. Wu, I.; Lin, J.; Cheng, E,2001. Acute Poisoning with the Neonicotinoid Insecticide Imidacloprid in N-methyl Pyrrolidone. *Clin. Toxicol.*2001, 39 (6), 617-621.

272. Yahia.E,(2016). Effandde certains perturbateurs Endocrine (pesticide) sur la reprodution chez les rat wistar. (These Doctorat). Departement de Biologie ; Faculte de science .Universite Annaba. p173

273. Youla A., Latrous Y.,2017., Contribution à l'etude phytochimique des flavonoïdes chez l'espèce (Melissa Officinalis L.) andevaluation de leur pouvoir antibacterien,.memoire de master 2,. Biologie andphysiologie vegetale,.Universite des Frères Mentouri Constantine,. 6 p.

274. -Zeghib A., (2013). Etude photochimique andactivites antioxydantes, antiproliferative, antibacterienne andantivirale d'extraits andd'huiles essentielles de quatre espèces endemiques du genre Thymus. Thèse de doctorat en sciences, chimie organique, Universite de Constantine 1, 2-10 p

275. Zhang J and Darley V (2000) Neurodegenerative Disease and Oxidative Stress 95-111

276. -Zheng, W.; Liu, W,1996. Kinetics and mechanism of the hydrolysis of imidacloprid. *Pestic. Sci.* 1999, 55, 482-485.

Annex

ANNEXES

1. Calibration curve for lipid determination

Tubes	1	2	3	4	5	6
Lipid stock solution (µl))	0	20	40	60	80	100
Ether / chloroform solvent (µl)	100	80	60	40	20	0
SPV reagent (ml)	2.5	2.2	2.5	2.5	2.5	2.5

Table 09: Creation of calibration curve for the determination of lipidsL'equation:

Y=0.0282857+0.0019843X

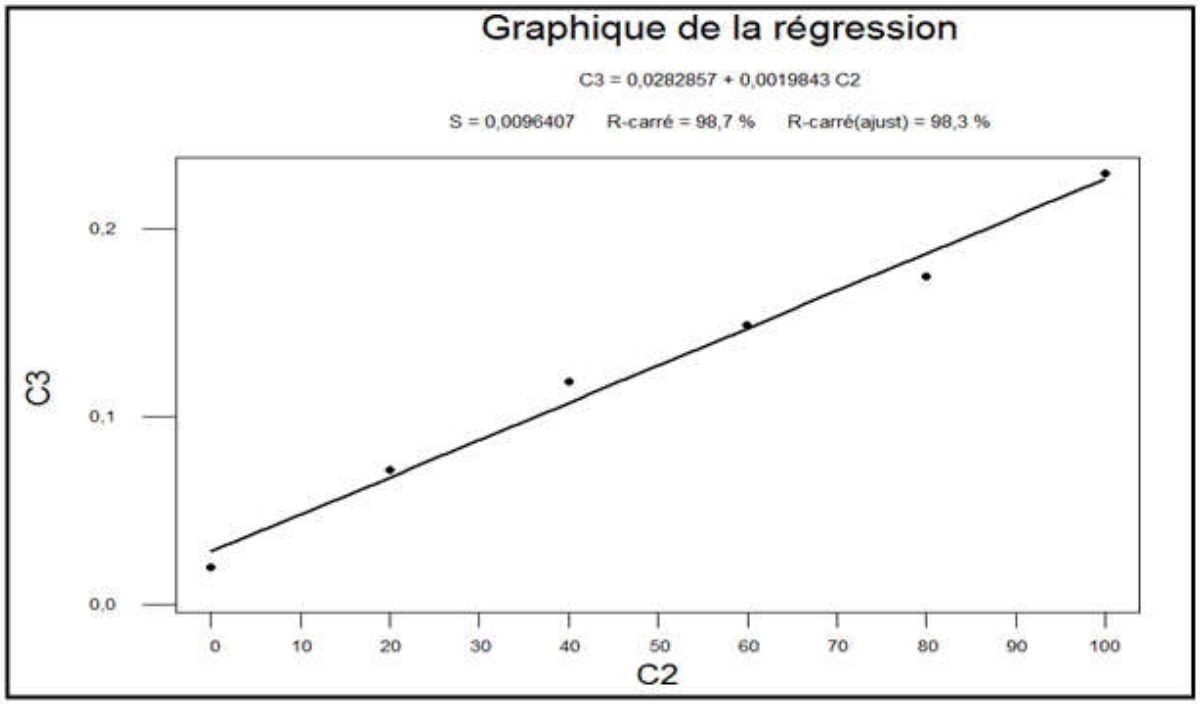


Figure (46). Creation of calibration curve for the determination of lipidsL'equation:

Y=0.0282857+0.0019843X

2. Calibration curve for protein assay

Tubes	1	2	3	4	5	6
Albumin stock solution (µl)	0	20	40	60	80	100
Distilled water (µl)	100	80	60	40	20	0
BBC reagent (ml)	4	4	4	4	4	4

Table (10). Calibration curve for protein assayL'equation**:** Y=0.135857+0.0083971X

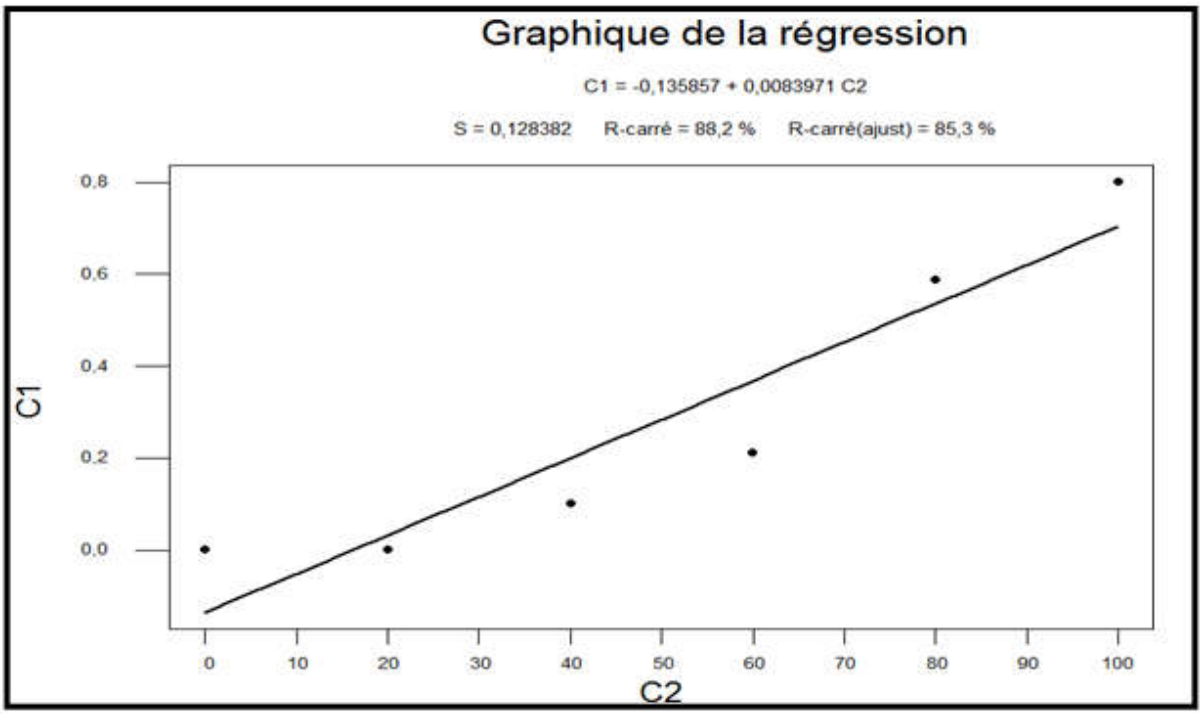
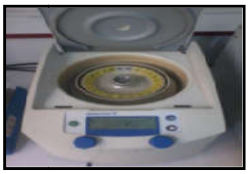


Figure (47). Calibration curve for protein assayL'equation**:** Y=0.135857+0.0083971X

3. Material used in the different stages of the study

Precision balance (KERN).



Centrifuge (SELECTA.

Water bath (MEMMERT).

Magnetic stirrer (WITEG).

Spectrophotometer (UV mini 1240, SHIMADZU).

Sand bath

Vortex (THERMOS).

- Fridge

Figure (48) : materiel of laboratory

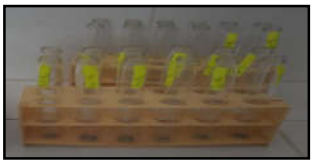
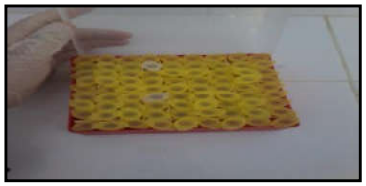
		
test tubes	Mortars	Glassware
		Wash bottle. Spatula. Magnetic bar. Magnetic rod extractor. End caps. - Cuvette for spectrophotometry (plastic and glass).
Micro-pipettes	epindorphic tubes	
100µl and 1000µl micropipettes. Pear. Graduated pipettes. Pasteur pipettes.	Racks for different types of tubes. Dry glass and plastic tubes.	Aluminium foil. Toilandpaper Beakers Erlenmeyers. - Graduated cylinders.

Figure 49: small laboratory equipment

4. Chemical material

- Distill water
- TCA (Trichloro acetique).
- Anthrone.
- Acide sulfurique.
- Acide orthophosphorique (à 85%).
- Vanilline.
- BBC (Bleu Brillant de Coomassie).
- Ether.
- Chloroforme.
- Ethanol (à 95%).
- BSA (Albumine serum de bœuf).
- Glucose.
- Sodium phosphate dibasique.
- SSA (Acide sulfosalicylique).
- Sodium phosphate monobasique.
- Tris.
- HCl.
- NaOH.
- Methanol absolu.
- EDTA (Acid ethylene diamine tetracetique).
- DTNB (acid 5-5'-dithio-bis-2-nitrobenzoïque).
- NaCl
- TBA
- BHT
- GSH
- CDNB (1-Chloro2, 4 di nitrobenzène)
- H_2O_2
- Phosphate
- Calcium Ca^{+2}