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Bioactivity of medicinal plants extracts against insect pests of stored grains

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Dedication



In the name of the most merciful the all mighty Allah

I dedicate this humble project to the most important man in my life., my father: ***NADJI BENSAIDANE***

And to my dear Queen my mother. I want to honor them for sacrificing and straggling so I can be the man I'm today

To my camarade ***AMINE BOUMENIR****., my mate who shared me the project and all my college years.*

And to my dear friends amine., sidali., kadour., younes., abdelbaki.,kamel., mourad., karim., adel.,yousef and ameur., I warmly thank them and ask God to bless and grant them all good things.

I dedicate my supervisor: ***Mm CHAOUATI KARIMA*** *who never stopped advising and motivating us during our search.*

And finally, I honor all those who helped me to finish this research ifen with a single word. I send them all my good wishes and hopes and may Allah grant them health and wealth.

BILLEL



Dedication



In the name of the most merciful the all mighty Allah

I dedicate this humble project to the most important man in my life., my father: ***MAHFOUD BOUMENIR***

And to my dear Queen my mother., I want to honor them for sacrificing and straggling so can be the man I'm today

To my camarade ***BILLEL BENSAIDANE****., my mate who shared me the project and all my college years.*

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I dedicate my supervisor: ***Mm CHAOUATI KARIMA*** *who never stopped advising and motivating us during our search.*

And finally, I honor all those who helped me to finish this research ifen with a single word. I send them all my good wishes and hopes and may Allah grant them health and wealth.

AMINE BOUMENIR

ABSTRACT

In the search for alternative solutions to chemical control based on the use of products bio-insecticide in order to combat *Tribolium castaneum* and *Callosobruchus maculates.*

The objective of this present study is to test the bio-insecticide effect of the various extracts of Atropa belladonna on adults of *Tribolium castaneum* and *Callosobruchus maculates.*

The overall analysis of the LD results shows that the effect induced on the survival of *C.maculatus* and *T.castaneum* varies considerably between the tested extracts of the species *A.Belladonna.*

we noted that the lowest concentrations that cause 50% mortality of *T. castaneum* adults are 0.14 mg for ethanolic root extract and 0.2 mg/ml for for the aqueous and ethanolic Leaves extract respectively the concentrations of the extracts that cause the mortality of 50% of adults of *C.maculatu*s., are 0.6 mg respectively for ethanolic Leaves extract and 0.72 mg/ml for the aqueous root extract. The extracts of the different organs of *A. Belladonna* showed very important insecticidal effects against *T. castaneum.*

The examination of LT_{50} shows that the adults of *T. castaneum* treated with the different extracts of *Atropa belladonna* have LT_{50} vary as a function of time. The highest LT_{50} is reached after 1.85 h for the ethanolic root extract. The highest LT_{50} for adults of *C.maculatus* is reached after 3.9 h for ethanolic root extract.

These results give a general idea about the bio-insecticide and to batter confirm our results we hope that our work will be followed by other complementary studies.

Keywords: Bio-insecticides, Aqueous extract, Ethanolic, Extract, LD_{50}, $LT_{50.}$

Résume

Dans la recherche de solutions alternatives à la lutte chimique, basées sur l'utilisation de produits bio-insecticides pour lutter contre *Tribolium castaneum* et *Callosobruchus maculates*.

L'objectif de cette présente étude est de tester l'effet bio-insecticide des différents extraits d'*Atropa belladonna* sur des adultes de *Tribolium castaneum* et *Callosobruchus maculates*.

L'analyse globale des résultats DL_{50} montre que l'effet induit sur la survie de *C.maculatus* et *T.castaneum* varie considérablement entre les extraits testés de l'espèce *A.Belladonna*.

nous avons noté que les concentrations les plus faibles qui causent la mortalité de 50% des adultes de *T. castaneum* sont de 0,14 mg pour l'extrait de racine éthanolique et de 0,2 mg/ml pour l'extrait de feuille aqueux et éthanolique respectivement les concentrations des extraits qui causent la mortalité de 50% des adultes de *C. maculatus*., sont respectivement de 0,6 mg pour l'extrait de feuille éthanolique et de 0,72 mg/ml pour l'extrait de racine aqueux. Les extraits des différents organes d'*A. Belladonna* ont montré des effets insecticides très importants contre *T. castaneum*.

L'examen de la TL_{50} montre que les adultes de *T. castaneum* traités avec les différents extraits d'*Atropa belladonna* ont une TL_{50} variable en fonction du temps. La TL_{50} la plus élevée est atteinte après 1,85 h pour l'extrait éthanolique de racine. La TL_{50} la plus élevée pour les adultes de *C. maculatus* est atteinte après 3,9 h pour l'extrait éthanolique de racine.

Ces résultats donnent une idée générale sur le bio-insecticide et pour mieux confirmer nos résultats nous espérons que nos travaux seront suivis par d'autres études complémentaires.

Mots clés : Bio-insecticides, extrait aqueux, extrait éthanolique, DL_{50}, TL_{50}.

الملخص

في البحث عن حلول بديلة للمكافحة الكيميائية ، بالاعتماد على استخدام منتجات المبيدات الحشرية البيولوجية للسيطرة على *Tribolium castaneum* و*Callosobruchus maclicates*

الهدف من هذه الدراسة هو اختبار تأثير المبيدات الحشرية البيولوجية و المستخلصات المختلفة من *Atropa belladonna*على الحشرات البالغة من *Tribolium castaneum* و *Callosobruchus maclicates*.

يُظهر التحليل الشامل لنتائج صعوبة التعلم أن التأثير الناجم على بقاء النوع *C.maculatus*و *T.castaneum*يختلف اختلافًا كبيرًا بين المستخلصات المختبرة من النوع *A.Belladonna*

لاحظنا أن أقل التركيزات التي تسبب 50 ٪ من وفيات البالغين *T.castaneum* هي 0.14 ملغ لمستخلص الجذر الإيثانولي و 0.2 ملغ/مل لمستخلص الأوراق المائية والإيثانولية على التوالي تركيزات المستخلصات التي تسبب وفيات 50 ٪ من البالغين من *C.maculatus* ، هي 0.6 ملغ على التوالي لمستخلص أوراق الإيثانوليك و 0.72 ملغ/مل لمستخلص الجذر المائي.
أظهرت مقتطفات الأعضاء المختلفة من *A.Belladonna* تأثيرات مبيدات حشرية مهمة جدا ضد*T.castaneum* .

يظهر فحص LT_{50} أن البالغين *T.castaneum* الذين عولجوا بمستخلصات مختلفة من *A.Belladonna* لديهم متغيرLT_{50} كدالة للوقت. يتم الوصول إلى أعلى LT_{50} بعد 1.85 ساعة لمستخلص الجذر الإيثانولي. يتم الوصول إلى أعلى LT_{50} للبالغين من *C.maculatus* 3.9 ساعة لمستخلص الجذر الإيثانولي.

تعطي هذه النتائج فكرة عامة عن المبيدات الحشرية البيولوجية ولتأكيد نتائجنا بشكل أفضل ، نأمل أن يتبع عملنا دراسات تكميلية أخرى.

الكلمات المفتاحية: المبيدات الحشرية الحيوية ، المستخلص المائي ، إيثانوليك المستخلص المائي ، دل 50، تل 50.

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List of Abbreviations

T°: temperature.

LD_{50} *:* lethal dose to kill 50% of insects.

LT_{50} : lethal time to kill 50% of insects.

Ext EtOH: ethanolic extract.

Ext H2O: aqueous extract.

AgNPs: Silver Nanoparticles

D: dose.

FTIR: Fourier-transform infrared spectroscopy.

SEM: Scanning Electron Microscopy.

TEM: Transmission electron microscopy.

Ste: stem.

Ro: roots.

Lea: Leaves

$AgNO_3$: Silver nitrate

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Introduction

Introduction

Stored foodstuffs occupy an important or majority part of the human diet., more precisely in developing countries (wheat., chickpeas., semolina., flour., barley., wheat bran., corn., corn flour., cereals ...)., in the form of stored grain or seeds. They are exposed to the attacks of pests that infest inside where they will feed multiply grow and secrete odorous sticky substances that contaminate all the stock in which they land.

Tribolium castaneum **(Herbst)** and *Tribolium confusum***(Jacqueline Duval)** are serious insects of products stored all over the world. **(Anonymous., in Schef & Arthur 2017)**. In Africa., *Callosobruchus maculatus* (Coleoptera: Bruchidae) is the harmful and dangerous insect that attacks and causes significant damage to legume stocks. Apart from cowpea which is its main host plant **(Weidner and Rack., 1984; Huiniard., 1985).**

In the search for alternative methods of control the plant kingdom offers many possibilities., and many studies are currently being carried out to isolate or identify substances extracted from plants that have repellent or anti-appetizing insecticidal activity against insects **(Licthenstein.,1996).**

In this context our work consists in evaluating the insecticidal potential of ethanolic and aqueous extracts of *Atropa Belladonna* species in the context of the valorization of natural plant substances.

Order to meet the objectives of this research. The work was divided into two parts;
A bibliographical part which includes a synthesis of the knowledge concerning *Atropa Belladonna*., the insect pest of chickpea the bruchus *Callosobruchus maculatus* and *Tribolium castaneum.*

An experimental part which is subdivided into two chapters: the methods and techniques used to carry out this work and the results obtained as well as the discussion. Finally, a general conclusion to highlight the main results obtained.

Part 1

Literature review

Chapitre I
Literature Review

I. *Atropa Belladonna L*

Belladonna (*Atropa belladonna*) is sometimes called Banewort., Belladonna., Bouton Noir., Chrottebeeri., Deadly Nightshade., Dway Berry., English Belladonna., Great Morel., Hexenkraut., Jijibe Laidour (Moroccan)., Mandragora., Poison Black Cherry., Sleeping Nightshade., Solanum Bacca Nigra., Tollkraut., Uva**(Cleversley.,2002).**

The etymology of the Latin name *Atropa Belladonna* breaks down as follows: the name of the genus «Atropa» comes from one of the 3 Fates or Moires of the Greek mythology., they are generally represented as spinners measuring the life of men and cutting destiny.Atropa corresponds to the name of one of them., "Atropos"., which in Greek means "inflexible"., which ruthlessly cuts the thread of life **(Zylberberg.,2014)**.The name of the species "Belladonna" comes from the Italian word for "beautiful lady". During the Renaissance., Italians used to instill belladonna juice in their eyes to dilate their pupils under the action of atropine., and thus give more attraction to their eyes**(Packer and Brandt.,1992).**

I.1. Place of *A.Belladonna L* in the classification

The name *Atropa belladonna* was published by **Carl Linnaeus** in Species Plantarum in 1753., its full scientific name is *Atropa belladonna L*. This plant has synonymous names which are **(Tela botanica.,2019):**

- Atropa acuminata Royle

- Atropa belladonna var. lutea Döll

- Atropa caucasica Kreyer

- Atropa lethalis Salisb.

- Belladonna baccifera Lam.

- Belladonna trichotoma Scop.

- Belladonna trichotoma Moench

It is still called in Latin Solanum somniferum., Solanum soporificum., or Solanum maniacum., Solanum that makes manic **(Stephenson and Churchill.,1834).**Belladone is named abroad and found in studies under the name of: Belladona (Spanish), Belladonna (Italian), Deadly Nightshade, Dwale or Raging Nightshade (English), Tollkirsche or Wolfsbeere

(German), Wolfskers (Dutch), Άτροπος η μπελαντόνα or Μπελαντόνα (Greek), Edge or Paret Tobacco (Catalan), Galnebær (Danish) **(Tela Botanica.,2019).**

Table 01: The classification of *Atropa Belladonna* According to **Integrated Taxonomic Information System (ITIS)**

Taxonomic rank	Nomenclature
Kingdom	*Plantae – plantes., Planta., Vegetal., plants*
Subkingdom	*Viridiplantae – green plants*
Infrakingdom	*Streptophyta – land plants*
Superdivision	*Embryophyta*
Division	*Tracheophyta – vascular plants., tracheophytes*
Subdivision	*Spermatophytina – spermatophytes., seed plants., phanérogames*
Class	*Magnoliopsida*
Super-order	*Asteranae*
Order	*Solanales*
Family	*Solanaceae – nightshades.,*
Genus	*Atropa L. – belladonna*

I.2. Morphological description of *Atropa Belladonna*

A.Belladonna is a perennial herbaceous plant with a sturdy, cylindrical stem that is delicately pubescent and glandular on the top portion by dichotomes and trichotomes and stands 50-150 cm tall **(Flora Helvetica.,2018).**The leaves are dark green, whole, little petiolated, oval, acuminate, that is to say, with a finely pointed tip. They alternate at the lower part of the stem, close by 2 and of uneven size at the upper part. The leaves are soft, finely pubescent, with foul-smelling veins **(Tela Botanica.,2019).**The calyx is campanulate, slightly hairy, persistent, with five acuminate oval lobes, with a slightly reflected tip. The corole is always gamopetal and regular, slender, whitish at the base, pleated with five short and obtuse lobes, and bell-shaped. Five stamens are inserted at the base of the corolla, each shorter than the other, with subulated threads that curve inwards and are slightly hairy at the base, and little cordiform anthers that

open lengthwise, mirrored on the threads following pollen emission. The ovary is ovoid and obtusely lobed, set on a yellowish disc, with a style longer than the stamens, a tiny, cylindrical stigma., and a somewhat bilobed, papillous style. The fruit is a berry surrounded at the base by the star calyx, the size of a small cherry, globose, slightly depressed, fleshy, first green, then red, finally a shiny black with two lodges containing many reniform seeds. The leaves are alternate, sometimes twinned upwards, few petioles, ample, oval, acuminate, whole., superficially sinuous, glabrous or pubescent, green, soft with slightly pale veins **(Costantini., 2019).**



Figure 01: The features of the *Atropa belladonna* plant showing the leaves, and berries **(Anonymous., 2021).**

I.3. Geographical distribution

I.3.1. Distribution of *Atropa Belladonna* in the world

Belladonna is widely distributed over Central and Southern Europe., South-west Asia and Algeria. It is cultivated in England, France and North America for medicinal purposes (**Grieve.,1931)**.

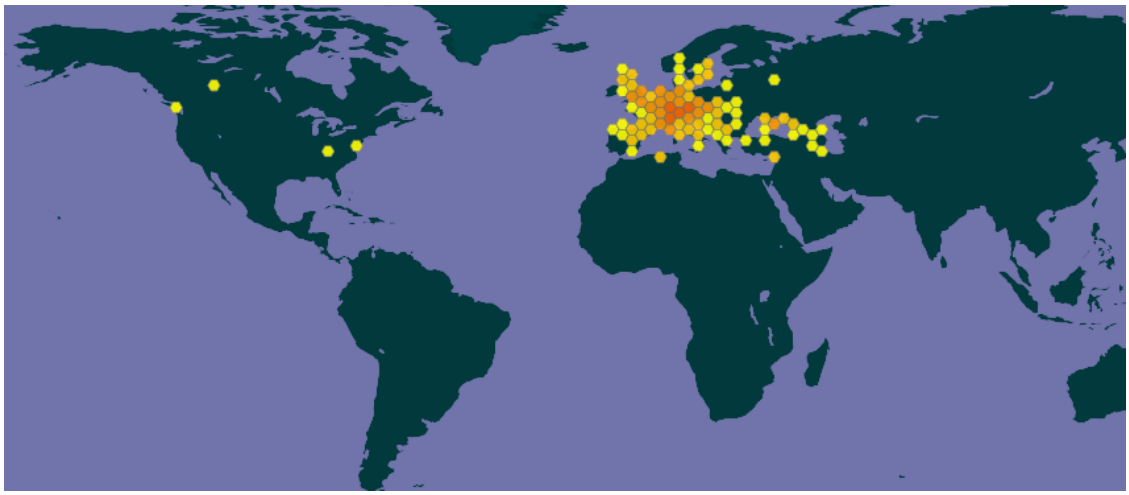


Figure 02: Worldwide distribution of *Atropa Belladonna*.

Source :(**GBIF Global Biodiversity Information Facility**).

I.3.2. Distribution of *Atropa Belladonna* in Algeria

Figure 03 shows the distribution of *Atropa Belladonna* in Algeria based on a human observation that appeared in inaturalist.

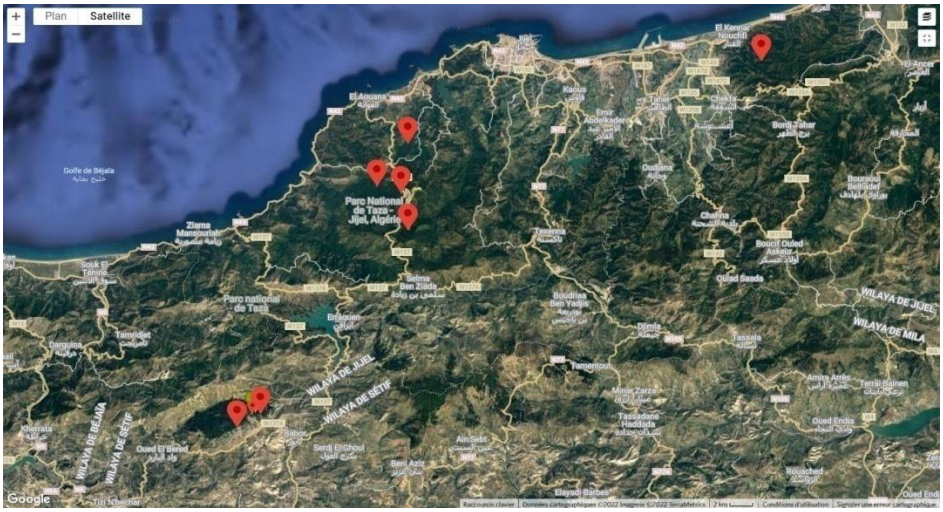


Figure 03: Distribution of *Atropa belladonna* in Algerian territory. **(Source: inaturalist.org).**

I.4. *A.Belladonna* biochemistry

I.4.1. General Information on alkaloids

Alkaloids are a heterogeneous chemical group of substances containing a basic nitrogen atom, found mainly in higher plants. These basic compounds have also been found in less evolved plants as well as in animals and microorganisms **(Sujata and *al*.., 2009; Sujata and *al*.., 2013).**

Alkaloids usually contain one or two nitrogen atoms but some can contain up to five. such as ergotamine. Contain up to five, such as ergotamine. The term alkaloid was coined in 1818 by the Meissner. It derives from "alkali-like" meaning that molecules of natural origin behave like alkalis. The exact term derives from the Arabic al kali, soda. and the Greek appearance **(Sujata., 2009; Sujata., 2013)(Bruneton.,2016).**

The nitrogen atom in the alkaloids usually comes from an amino acid whose carbon structure often remains intact in the final structure of the alkaloid. Thus, there are alkaloids derived from Ornithine, Lysine, Tyrosine, Phenylalanine and Tryptophan **(Mauro., 2006).**There are different types of alkaloids,these include tropanic alkaloids **(Facchini.,2001).**

I.4.2. Functions of plant alkaloids

The alkaloids are molecules resulting from the secondary metabolism of the plant and their presence is justified by three main functions:

• Poisons protecting plants against insects and herbivores, such as hyoscyamine, atropine and

hyoscyamine, atropine and scopolamine in belladonna.

• End products of detoxification reactions representing a metabolic blockage of compounds compounds those are sometimes harmful to the plant.

• Réserves de substances capables de supplémenter en azote ou autres fragments nécessaires au développement de la plante **(Sujata., 2009; Sujata., 2013).**

I.4.3. Identification and general qualitative tests

Alkaloids are generally bitter tasting and optically active. This property is very useful for very useful for the identification of racemic acids. The alkaloid bases are mainly non-volatile crystalline solids non-volatile crystalline solids, insoluble in water and soluble in organic

solvents polar solvents (methanol, chloroform, ether), nicotine is soluble in water.Almost all alkaloids form salts with inorganic acids making them soluble in water **(Sujata., 2009; Sujata., 2013).**

Alkaloids give characteristic precipitates and colors in the presence of known reagents: cream-colored precipitate in the presence of the Valser-Mayer reagent (potassium mercury iodide), reddish brown or pinkish with Wagner's reagent (iodine solution) and red-orange precipitate with Dragendorff's reagent (acidic solution of potassium iodobismuthate).The precipitate can be of crystalline or amorphous structure **(Bruneton., 2016).**

I.4.4. Tropanic alkaloids

Tropanic alkaloids are a class of alkaloids characterized for the presence of a tropane nucleus, a cyclic nitrogen compound. More than 200 tropical alkaloids are known worldwide. Mainly 3 families of plants synthesize these tropanic alkaloids: Solanaceae, Erythroxylaceae and Convolvulaceae **(Christen., 2000).**

Atropine., cocaine and scopolamine are the three most common tropanic alkaloids in plants **(Aniszewski.,2007)**, they are extracted from various solanaceous plants belonging to the genera Atropa., Duboisia., Datura and Hyoscyamus**(Mateus and *al*.,2000).**Tropanic alkaloids all derive from a common nucleus the (1R.,5S) tropane structurebicyclic condensing pyrrolidine and piperidine cycle: it is N-methyl-8-azabicyclo octane adopting a chair-like spatial configuration **(Paul.,2009;Mothes and *al*.,1985).**

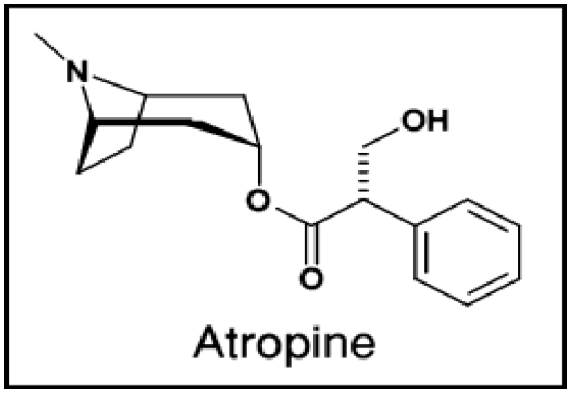


Figure 04: Atropine nucleus **(Yahia, M., 2018).**

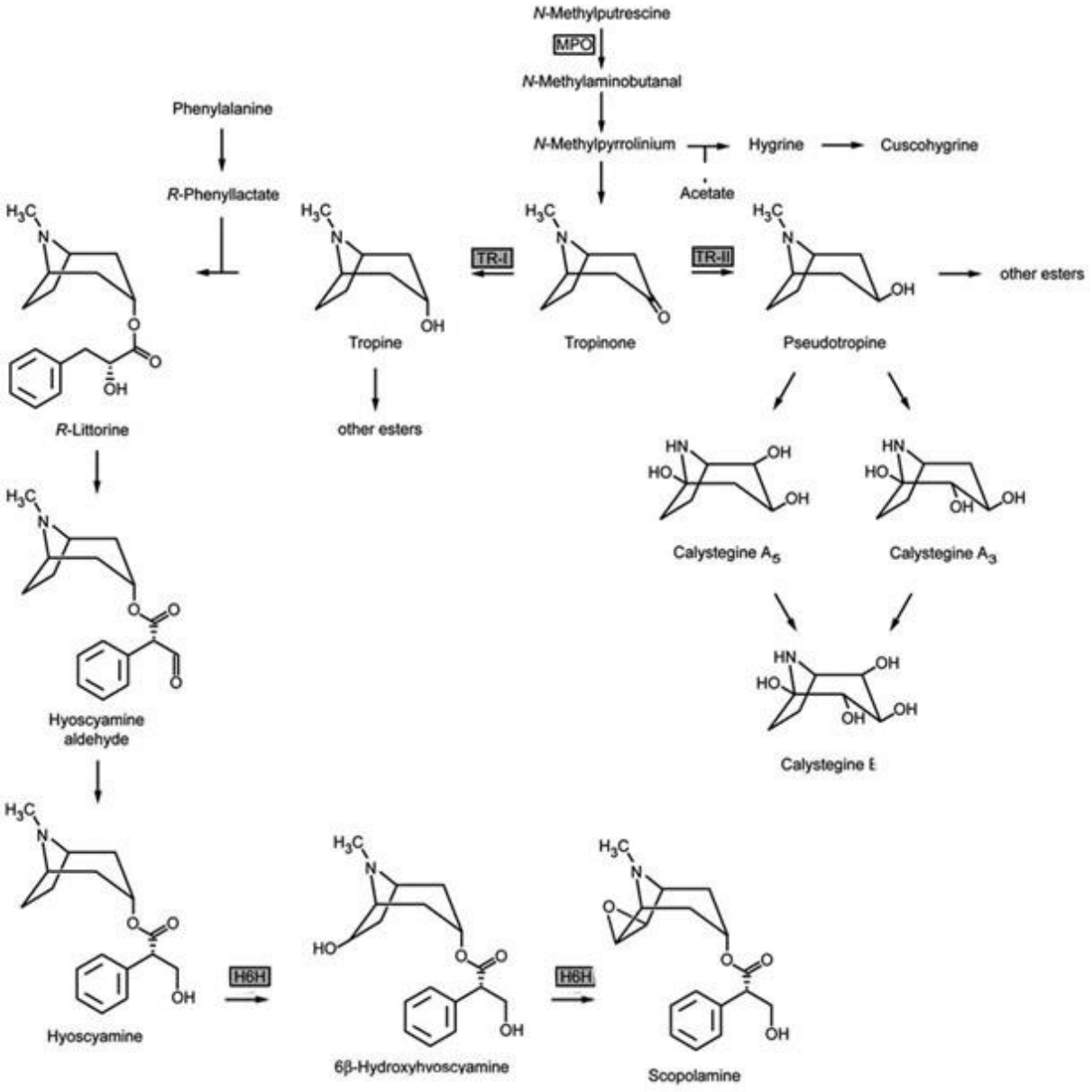


Figure05: Tropanic alkaloid biosynthesis pathway (**Oksman., 2007).**

A- Atropine chemistry

The name atropine was coined in the 19th century, when pure extracts from the belladonna plant Atropa belladonna were first made **(Goodman and Gilman's., 2001).**

In 1809, the French chemist isolated an impure form from belladonna. In 1822, Rudolph Brandes, a German pharmacist, named this active molecule atropine. This active molecule was named atropine by Rudolph Brandes, a German pharmacist, But the pure atropine seems to have been isolated the first time by a German pharmacist named A. Mein (1790-1850) from dry roots of belladonna in 1831**(Arquinet.,2019).**

Atropine is a racemic mixture of: (S)-hyoscyamine naturally present in plants and endowed with a high pharmacological activity as well as (R)-hyoscyamine which appears during extraction and which has a lesser activity. Young organs contain practically pure S-(–)-hyoscyamine while older organs are characterized by the presence of R-(+) isomer **(Arquinet., 2019).**

B- Hyoscyamine Chemistry

Hyoscyamin is a direct precursor in plant biosynthesis of hyoscin and is produced by the same metabolic pathway.

Biosynthesis begins with decarboxylation of L-ornithine to putrescine by ornithine decarboxylase. Putrescin is methylated to N-methylputrescin by putrescin Nmethyltransferase**(Ziegler.,2008).**

A putrescin oxidase that specifically recognizes methylated putrescin catalyzes the deamination of this 4-methylaminobutanal compound which then undergoes spontaneous cycling in N-methylpyrrolium cation. In the next step, the pyrrolium cation condenses with acetoacetic acid to give hygrine. No enzymatic activity could be demonstrated. Then the hygrine rearranges into tropinone. Subsequently, the tropinone-reductase I convert tropinone into tropine which condenses with phenyllactate derived from phenylalanine into littorine. A cytochrome P450 classified Cyp80F1 oxidizes and rearranges the littorin into aldehyde hyoscyamin., then an enzyme, dehydrogenase, then reduces the aldehyde into one primary alcohol thus forming the hyoscyamine **(Ziegler.,2008).**

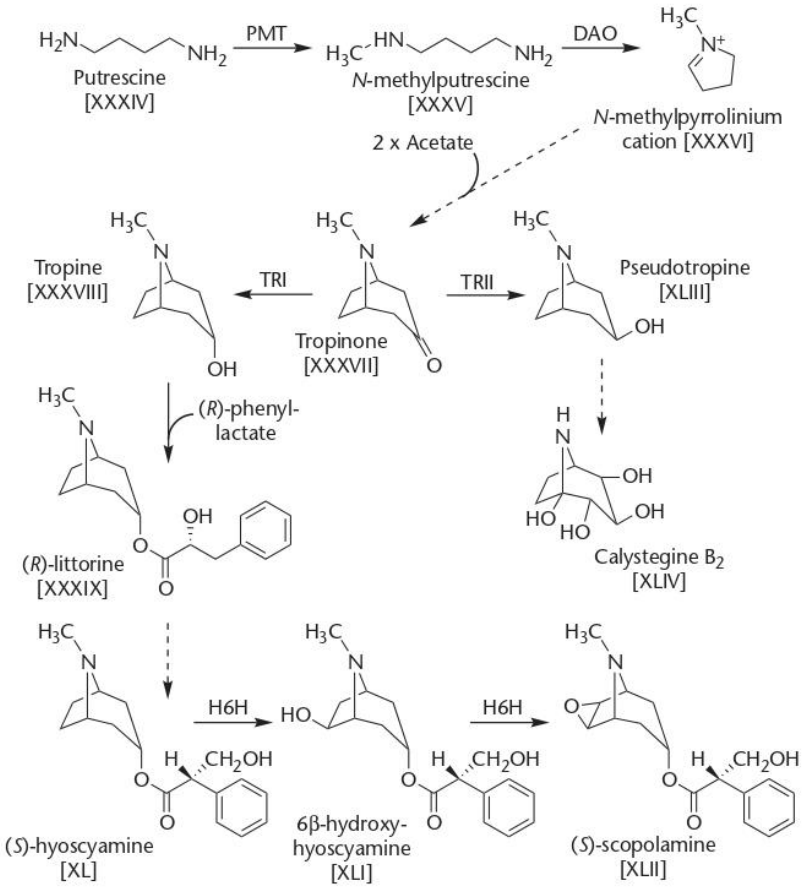


Figure06: Biosynthesis of the tropane alkaloids **(Fester., 2010).**

C- Scopolamine chemistry

Scopolamine ou hyoscine est le 6.,7-β-époxyde l'hyoscyamine. It is formed from hyoscyamine using 6β-hydroxyhyoscyamine.Hyoscyamin 6β-hydroxylase (H6H) dioxygenase dependent on 2-oxo-glutarate catalyzes the hydroxylation of hyoscyamin to 6βhydroxyhyoscyamine and the epoxidation of 6β-hydroxyhyoscyamine to scopolamine **(Zhangand *al*., 2004).**

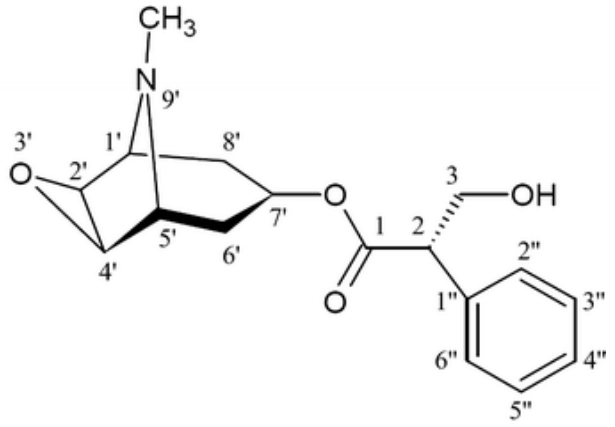


Figure 07: Molecule representing scopolamine **(Ullrich., 2017).**

I.1.5. Quantity of components in the various organs

There are 13 distinct tropanic alkaloids in the roots and 7 in the aerial parts**(Hartmann and *al*.,1986)**.The proportion of total alkaloids in the leaf varies depending on when it was harvested and whether the plant is wild or cultivated., the latter being richer alkaloid. Alkaloid concentration is between 0.3% and 1% dry plant. Atropine and L-hyoscyamine represent 90-95% of total alkaloids and scopolamine 5-10 % **(Goullé and *al*.,2004).**

The whole plant is toxic, but especially the roots and fruits.there are also some minor alkaloids derived from the tropane nucleus. Namely tigloyltropein, aposcopolamine, apoatropine, hydroxy-hyoscyamine and tigloyoxytropane, as well as a pyrrolidine alkaloid called hygrine.**(Arquinet., 2019).**

Table 02: Total alkaloid levels in the various organs of Atropa belladonna. Some bibliographic references. **(Arquinet., 2019)**

Source	Molecules	Root	Leaves	Fruit
(Ciccone)	Total alkaloids	0.85%	0.50%	0.65%
(To1xiplante)		0.85%	0.50%	0.65%
(Rey-Giraud 2018)		0.7 à 0.9%	0.3 à 0.6%	0.65%

II. Nanotechnologie

"Nanotechnology" is an umbrella term that describes applications in many scientific fields but generally covers research on principles and properties existing at the nanoscale. The goal of nanotechnology consists of producing objects or materials smaller than 100 nanometers. That is bring together everything related to design., characterization., production and application structures., devices and systems by controlling shape and size at scale nanoscale. Nanotechnologies are part of applied research. Applications concerned have significant economic potential in many areas such as than medicine, chemistry, energy, resource saving or the environment **(Belkacem.,2015).**

II.1. Nanomaterials

On 18 October 2011, the European Commission publishes its recommendations onto the definition of nanomaterials (Recommendation 2011/696/EU): "nanomaterial" means a natural,

accidentally formed or manufactured material containing free particles., in aggregate or agglomerate form, of which at least 50 % of the particles, in the numerical size distribution, have one or more dimensions external between 1 nm and 100 nm". **(Belkacem.,.2015)**There are two main families of nanomaterials

- **Nano-structures**
- **Nano-objects**

II.1.1. Nano-objects

Nano-objects are materials with one, two or three external dimensions in the field nanoscale. Among these nano-objects, nanoparticles include their three dimensions in the nanometric domain, nanoplates have a nanometric dimension while nanofibers are nano-objects presenting two dimensions **(Gaffet., 2011).**

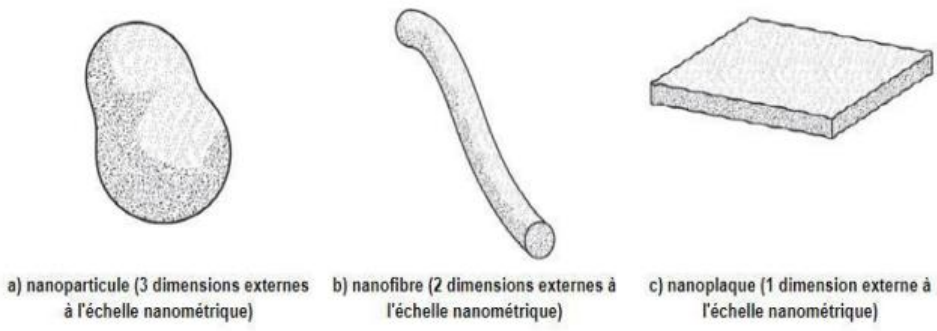


Figure 08: The three main forms of nano-objects.

II.2.Silver nanoparticles

Since 1994., nanomaterials based on silver nanoparticles have developed exponentially by covering a large number of industrial sectors: materials antibacterial **(H.Q. Jiang., S. Manolache 2004) ,**antistatic materials, cryogenic superconducting materials**(Hirano ,S and *al* 2004)** ,biosensor materials**(Ren and Tang.,2002)**, synthetic textiles**(Yeo and *al* ,. 2003)**, printed circuit boards**(Wang and *al*., 2005)**, materials catalytic**(Zhang and *al*., 2004).**

Historically, the synthesis of silver nanoparticles is carried out in the laboratory by reverse micellar route **(Petit and al,. 2004).**

The metal-organic salt reduction method was used by Zheng and Stucky.,to synthesize gold nanoparticles **(Zheng and al,. 2006).** The gold salt precursor ClAuPPh3 is reduced bytert-

butylamine borane (TBAB)., in the presence of dodecanethiol., at a temperature of reaction of 100 °C. Tang and al. Have performed according to an equivalent procedure., the synthesissilver nanoparticles **(Tang., 2007).**

II.2.1. Silver

Silver is a soft. White, shiny transition metal and a chemical element with the number atomic 47. Symbol Ag. Among copper and gold. Silver belongs to the column of the periodic table and is one of the most heat and electricity conducting metals. It is used in many forms such as colloids as lotions. It is the main therapeutic agent in medicine for diseases infectious diseases and surgical infections **(Firdhouse., 2015).**

II.2.2. Properties of Silver Nanoparticles

AgNPs have distinctive physico-chemical properties including high electrical and thermal conductivity., chemical stability., catalytic activity and non-linear optical behaviour **(Tran et *al.*, 2013).**More mechanical and magnetic properties compared to bulk materials**(Renata and *al.*, 2012)** of which:

A- Optical properties

AgNPs are extremely effective for absorbing and diffusing light and., unlike many dyes and pigments have a color that depends on the size and shape of the particle **(Elham et *al.*, 2016).**

B- Surface chemistry

When the nanoparticles are in solution, the molecules combine with the surface of the nanoparticles to establish a double layer of charge that stabilizes the particles and prevents aggregation **(Elham et *al.*, 2016).**

C- Biological properties

Silver compounds are shown to be effective against aerobic and anaerobic bacteria by precipitating bacterial cell proteins and blocking the microbial respiratory system **(Elham et al., 2016).**

II.3. Biological Synthesis of Silver Nanoparticles

The green synthesis of nanoparticles is gradually emerging as a key branch of nanotechnology in which nanoparticles are produced using biological entities **(Prateek et *al*., 2017).,**where negatively charged cell wall electrostatically interacts with positively charged and bio charged metal ions reduces metal ions in NPs**(Tran et *al*., 2013).**

This method can be considered as an environmentally friendly approach and also as a low-cost, high-efficiency, non-toxic technique **(Soliman et *al*., 2018).** simple, reliable **(Rajeshkumar and Bharath., 2017),** high density, stability and immediate solubility of prepared nanoparticles in water**(Zhang et al., 2016).**In relation to chemical synthesis methods and physics that indicate low biocompatibility and latent biological risks consume important energy, require high pressure, expensive and have low return**(Mohamed et *al*., 2019).**

Table 03: Comparison of Biological and Chemical Synthesis **(Muhammad et *al*., 2016).**

Nanoparticle synthesis		
Bottom-up approche		Top-down approche
Green methods	Chemical methods	Physical methods
-Use bacteria. -Use fungus. -Use plants and their extracts. -Use yeast. -Use of enzymes and biomolecules. -Use a microorganism	-Chemical reduction -Sonochemical -Microemulsion -Photochemical -Electrochemical -Pyrolysis -Microwave oven -Solvothermal	-Pulsed laser ablation. -Evaporation-condensation. -Arc Tip. -Pyrolysis by spraying. -Ball milling. -Steam and gas phase. -Pulse wire discharge. -Lithography.
Nontoxic	Toxic	

Due to its relatively versatile nature secondary metabolite extracts collected from biological sources contain many biomolecules such as enzymes, proteins, flavonoids, sterols, triterpenes, triterpenoids and saponins they have reductive propertiesand antioxidants responsible for reducing metal compounds in their respective nanoparticles **(Keerawelle and Chamara., 2019).**The following are some of the reducing and/or stabilizing agents used in the green synthesis of silver nanoparticles:

II.3.1. Bacteria

The use of bacteria is rapidly gaining importance due to its increasing success., ease of handling and genetic modification AgNPs have been synthesized using both gram-positive bacteria and gram-negative to form these silver nanoparticles, it should be known that some

bacteria have the ability to produce intracellular nanoparticles while others produce extracellular AgNPs **(Chauvel., 2018).**

Bacteria commonly use nitrate as the primary source of nitrogen through which nitrate is converted to nitrite by nitrate reductase, using the reductive power of a reduced form of nicotinamide adenine dinucleotide (NADH). In fact, the use of nitrate reductase as a reducing agent is proving to play a key role in the bioreduction of Ag+ ions **(Sang Hun and Bong-Hyun., 2019)**

II.3.2. Fungus

Fungi have potential to synthesize metal NPs due to the bioaccumulation capacity and tolerance of metals., their high binding capacity and intracellular absorption which are easy to handle in a research facility in relation to bacteria., also they secrete huge enzymes catalyzing the reduction of AgNO3 solution which tends to significantly increase the profitability of this synthetic approach **(Muhammad et *al.*, 2016).**

The mechanism of Fungus-based synthesis of AgNPs according to **(Muhammad et *al.*, 2016):**

a) Ag+ particles were adsorbed to the surface of the fungal cells due to the presence of electrostatic interaction between negatively charged carboxylic groups in enzymes and Ag ions positively charged**.**

b) Ag particles reduced by enzymes in cell walls causing the development of Ag nuclei.

c) Nanoparticles form on the surface of mycelium, not in solution.

II.3.3. Polysaccharide

In this method, AgNPs are prepared using water as an eco-friendly solvent and polysaccharides as a capping agent and reducer. In a gently heated system of starch and β-glucosis (reducing agent), the formation of AgNPs is obtained.

This solution, combined with AgNO3 in salt form allows .The synthesis of 10-34 nm silver nanoparticles. These AgNPs are stable in the aqueous solution at 25°C for about 3 months **(Chauvel., 2018).**

II.3.4. Plants

Plants are considered an extensive method for the rapid synthesis of silver nanoparticles due to their wide spectrum of bioactive secondary metabolites, such as polyphenols, flavonoids, terpenoids, amino acids, proteins and enzymes, which could improve the synthesis of nanoparticles, playing a stabilizing or reducing role **(Chauvel., 2018).**

Plant parts such as roots, stem, seeds and leaves are mainly used for the synthesis of NPs. The interesting point is the active agent present in these parts **(Muhammad et *al*., 2016).**

The bio-reduction of Ag has been postulated as trapping Ag+ ions on the surface of proteins because of the electrostatic interactions between these two in the extract of plant material. Proteins reduce Ag+ ions, resulting in their secondary structural change and the formation of silver nuclei which develop successively by additionnel réduction of Ag+ ions and their accumulation at the nuclei leading to the formation of AgNPs **(Rajeshkumar et Bharath., 2017).**

After selection of the plant extract.The main affecting parameters are the concentration of the extract, temperature, metallic salt, pH and contact time **(Muhammad et *al*., 2016).**

II.4. Characterizations of silver nanoparticles

After synthesis. characterization of AgNPs is essential to study their characteristics such as surface, morphology, size, shape, aggregation and solubility, etc **(Rajeshkumar et Bharath., 2017).**

II.4.1.UV-Visible Spectroscopy

UV-visible spectrophotometry is one of the simple, easy, fast, sensitive and most commonly used techniques for the characterization of synthesized nanoparticles. This involves quantifying the amount of ultraviolet or visible radiation absorbed by a constituent in solution. And measures the ratio of the intensity of two beams of light in the UV region Visible **(Rajeshkumar et Bharath., 2017).**

In the AgNPs, the conduction band and the valence band are very close to each other in which the electrons move freely. These free electrons give rise to a Surface plasmon resonance (SPR), due to the collective oscillation of silver nanoparticle electrons in resonance with the light wave **(Zhang et *al*.., 2016).**

II.4.2. Fourier-transform infrared spectroscopy (FTIR)

FTIR is a non-invasive, adapted, valuable, cost-effective, and simple way to study the role of biomolecules in the reduction of AgNO3 in silver.

When infrared radiation passes through the sample results. Certain of this radiation is absorbed by the sample and remains passed through. The resulting spectrum means absorption and transmission by creating a molecular foot print of the sample that represents its identity **(Rajeshkumar et Bharath., 2017).**

II.4.3. Scanning Electron Microscopy (SEM)

This is a commonly used technique, as the handling of this type of equipment is quite common, the resolution of which remains quite good, varying between 1 and 7 nm. In addition to the observation of the microstructure, it makes it possible to identify the chemical composition at the local and even global scale of the sample, via the EDX analyzer, incorporated in the apparatus **(Belfennache., 2010)**

II.4.4. Transmission electron microscopy (TEM)

The (TEM) is a fundamental tool for the study of materials at the nanoscale. Used in conventional mode, which determines the morphology, size, polydispersity and crystallinity of the synthesized nanoparticles, it is a very performant technique, with a much better resolution than the SEM, among 0.1 and 1 nm. However its handling remains delicate **(Belfennache., 2010).**

III. Plant Secondary Metabolites

Secondary metabolites are low molecular weight compounds (<1000 Da generally) which don't fulfil their fundamental role for plants such as primary metabolites, but which are involved in the adaptation of plants to their environment., especially in biotic interactions. **(Ramakrishna and Ravishankar., 2011; Naik and al-Khayri., 2016).** In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites (e.g., carbohydrates, lipids and amino acids) and are the result of co-evolution between plants and their biotic environment. Known as active ingredients for pharmaceutical industry, Food additive and Flavoring or perfumes.,It also contributes to the specific odors, tastes and colors in plants.**(Ramakrishna and Ravishankar.,2011 ;Murthy and *al.*., 2014)**. Their role in the

growth and development of plants is increasingly being studied., particularly to improve cultivation techniques, for example they could provide plants with survival benefits by protecting them from biotic or abiotic stresses, improving nutrient availability, acting as a metabolic defense mechanism, or improving competitive interactions with other organisms. **(Breitling and *al*..,2013).** Secondary plant metabolites are divided into 3 chemical groups: alkaloids, terpenoids and phenolic compounds. Among them, polyphenols constitute a vast diversity of bioactive compounds involved in many processes of biotic interactions from symbiosis to disease resistance. **(Cheynier and *al*..,2013).** They are defined as "non nitrogenous derivatives of which the aromatic ring or rings are primarily derived from the metabolism of shikimic acid or/and polyacetate" **(Bruneton.,2009).**

III.1. Biosynthesis of secondary metabolites

Secondary metabolites are generally the result of three biosynthesis pathways: shikimate pathway, mevalonate pathway and pyruvate pathway.**(Verpoorte and Alfermann.,2000)**

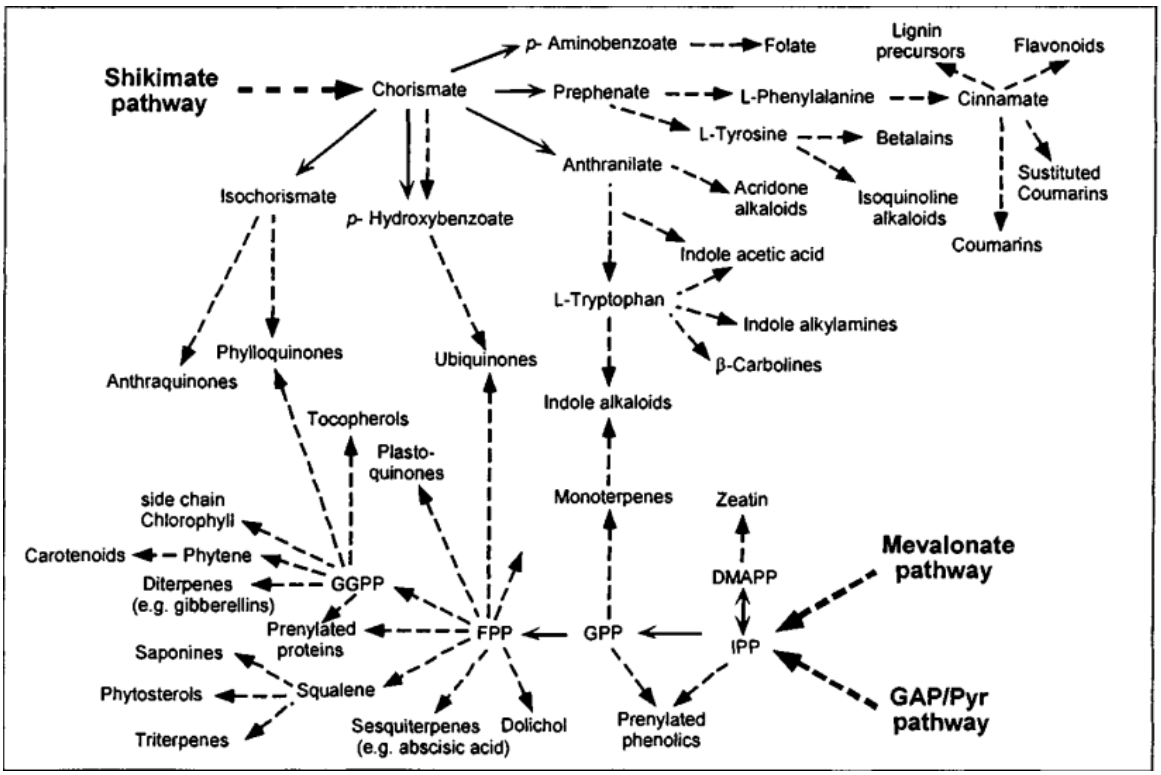


Figure 09: Terpenoid and shikimate pathways, two major routes leading to various secondary metabolites. **(Verpoorte and Alfermann., 2000).**

III.2. Classification of secondary metabolites

Secondary metabolites are produced in extremely low quantities., there are more than 200000 secondary metabolites classified according to their chemical belonging in this case., the terpenes, alkaloids, acetylene compounds, waxe., and phenolic compounds.**(Vermerris., 2006).**There are three major classes:

III.2.1. Phenolic compounds

Polyphenols are secondary metabolites present in all vascular plants.(**Lebham., 2005).**They are present in all parts of the higher plants(roots, stems, leaves, flowers, pollens, fruits, seeds and wood),and are involved in many physiological processes such as cell growth, rhizogenesis, seed germination and fruit maturation**(Boizot and Charpentier., 2006).**

They group together a large range of more than 8.000 molecules divided into a dozen chemical classes, all of which have one thing in common: the presence in their structure of at least one 6-carbon aromatic ring, itself carrying a variable number of OH hydroxyl functions. **(Hennebelle., 2004).**They result from two main synthetic pathways: the shikimate and acetate pathway**. (Lugasi et *al*., 2003).**

Phenolic compounds (phenolic acids, flavonoids and Proanthocyanidin) form the most important group of plant phytochemicals. **(Beta et *al.*, 2005).**

II.2.1.1. The main classes of phenolic compounds

Phenolic compounds are classified according to the number of carbon atoms in basic structure. These structures can be free form or bound to the ester or Glycoside. **(Bruneton., 1999).**The different classes of these phenolic compounds and the plants containing them are represented in Table 03.

Table 04: Main classes of phenolic compounds **(Bruneton., 1999 ; Hennebelle., 2006).**

Number of Carbon Atoms	**Basic structure**	**Class**	Exemples	Plants
6	C6	Simple phenols	Catechol., Hydroquinone	Bearberry
7	C6-C1	Phenolic acid Benzoic	Gallic acid.,Salicylic acid.,Vanillin	Artichoke
8	C6-C2	Acetophenone	3-Acetyl-6-methoxybenzaldehyde	Willow
9	C6-C3	Phenolic acid Cinnamic	P-Coumaric acid Caffeic acid	Rosemary horse chestnut
10	C6-C4	Naphthoquinone	Shikonine	Drosera
13	C6-C1 C6	Xanthone	Bellidifolin., Mangostin	Root of Gentian.,Centaurea
14	C6-C1 C6	Stilbenes	Hydrangenol., Pinosylvin	Grape.,Pine
15	C6-C1 C6	Flavonoid Isoflavone	Quercetin	Ginkgo Thym camomille
18	(C6-C3)2	Lignan	Matairesinol	Thistle
30	(C6-C3-C6)2	Biflavonoid	Amentoflavone Hinokiflavone	Garcinia Hypericum
N	(C6-C3-C6)n	Condensed tannin (Proanthocyanidins)	Aesculitannins	The Horse Chestnut.,vine

A- Phenolic acid

These acids are contained in a number of agricultural and medicinal plants **(Psotová and *al*..,2003)**.Phenolic acids are present in abundance in food and are divided into two classes: benzoic acid derivatives and cinnamic acid derivatives. Hydroxycinnamic acids are more common than hydroxybenzoic acids and essentially include p-coumaric, caffeine, ferulic and sinapic acid. **(Pandey and Rizvi., 2009).**

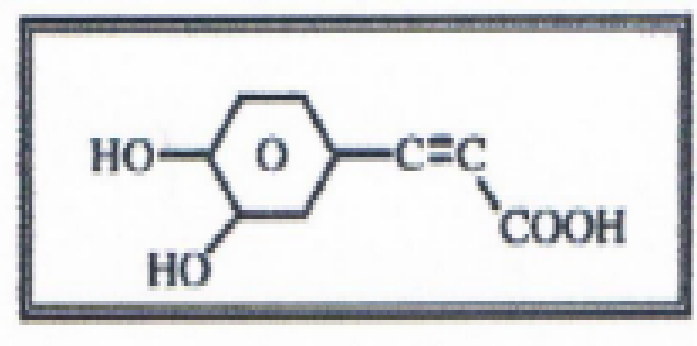


Figure10: Structure of caffeic acid **(Cowan., 1999).**

B- Tannins

Tannins are polar polyphenols from vegetable source., exist in almost every part of the plant :bark., wood, leaves, fruits and roots,their molecular weights range from 500 to 3000**(Cowan., 1999)**.It is difficult to separate them into plant extract.., because many very similar molecular-based isomers co-exist.**(Berthod et *al.*, 1999)**.

- **Chemical structure and classification**

Tannins are divided into two categories based on their chemical structure and synthesis pathway: Condensed tannin and Hydrolysable tannin**(Makkar., 2003).**

- **Condensed tannin (proanthocyanidins)**

More complex structure,calledproanthocyanidins,widely present in the plant kingdom and found in many food products (fruit, vegetables, beverages, etc.)**(Peronny., 2005),** they do not contain sugars in their molecule.,and not hydrolyzed by acids or tannins but in the presence of strong acids or oxidizing agents.,they are transformed into red substances: phlobaphenes **(Atefeibu .,2002).**

- **Chemical structure and classification**

These are polymers of flavan -3 ols., also called catechins and flavan - 3,4-diols called Leucoanthocyanidinor a mixture of both(**Atefeibu., 2002 ; Peronny., 2005)**.

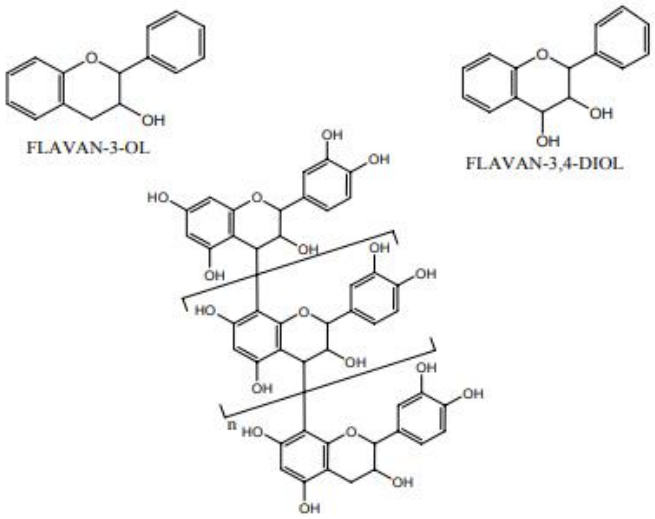


Figure 11: Structure of condensed tannins **(Peronny., 2005).**

- **Hydrolysable tannin**

These are glucose esters; they are characterized by the fact that they can be degraded by chemical or enzymatic hydrolysis. They then release a non-phenolic part (most often glucose or quinic acid) and a phenolic part that may be gallic acid. **(Macheix and *al*., 2005).** They consist of a central nucleus -glucose- and side chains (position 1., 2., 3., 4 or 6 on glucose) comprising 1 to n phenol acid monomer(s) carbon to carbon bonds between nuclei (biphenyl bonds produced by oxidative coupling), lead to more rigid collected molecules of reduced solubility known as elylagic tannins. **(Bessas and al., 2007).**

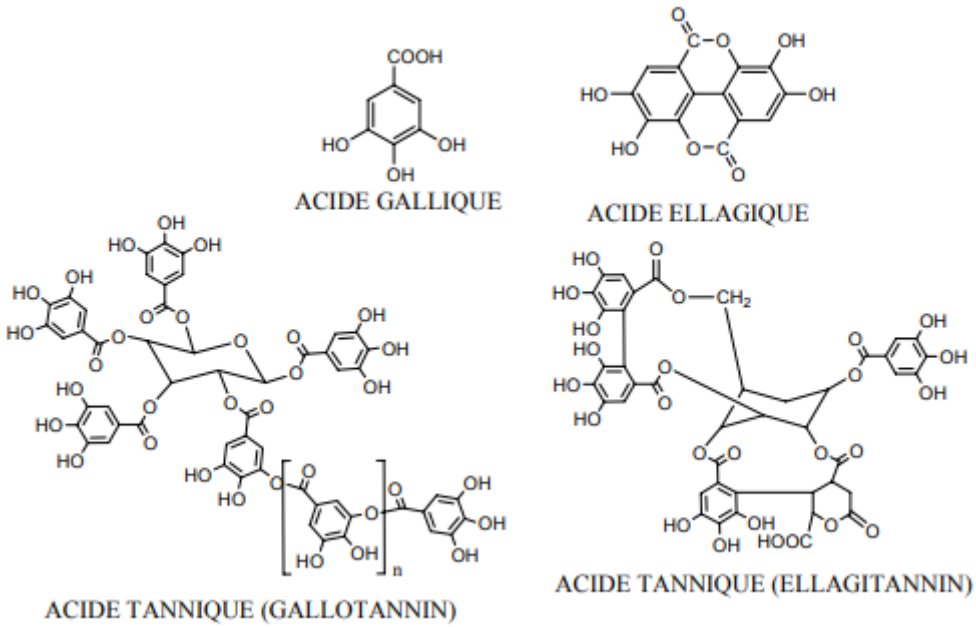


Figure 12: Structure of hydrolysable tannins and associated acids **(Peronny., 2005).**

C- Flavonoids

Flavonoids have a basic skeleton of 15 carbon atoms consisting of two phenyl cycles,cycles A and B., linked by a three-carbon chain (C6-C3-C6 structure).The C3 chain between cycles A and B is commonly cycled to form cycle C**(Bougandoura., 2011).** Flavonoids are able to exercise in addition to antioxidant properties, anti-inflammatory, anti-allergic and antiulcerogenic properties. Some flavonoids have also shown vasodilatory potential. They have been nicknamed the «naturalmodifiers of biological responses» **(Bruneton., 1993).**

- **Chemical structure and classification**

Flavonoids are benzo-γ-pyran derivatives **(Skerget and *al* ., 2005).**Their basic structure is that of a diphenyllpropane with 15 carbon atoms (C6-C3-C6).consisting of two aromatic nuclei (or rings) designated by the letters A and B ,connected by an oxygenated heterocycle, which denotes the letter C.**(Dacosta., 2003).**

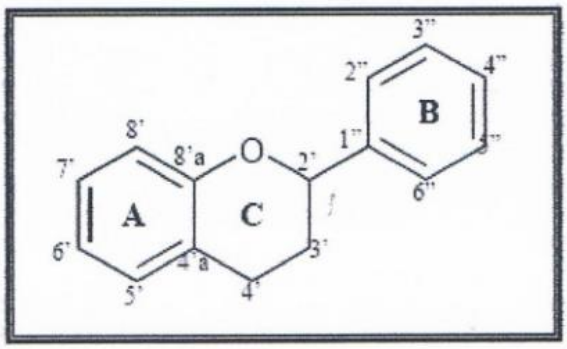


Figure 13: Basic skeleton of flavonoids **(Girotti Chanu., 2006).**

Flavonoids are generally found either in the free state. In this case they are called aglycones, either in the form of C- or O-glycosides, and in this case they are bound to sugars suchas glucose, rhamnose, arabinose, they may also be monomers or oligomers **(Dacosta., 2003).**

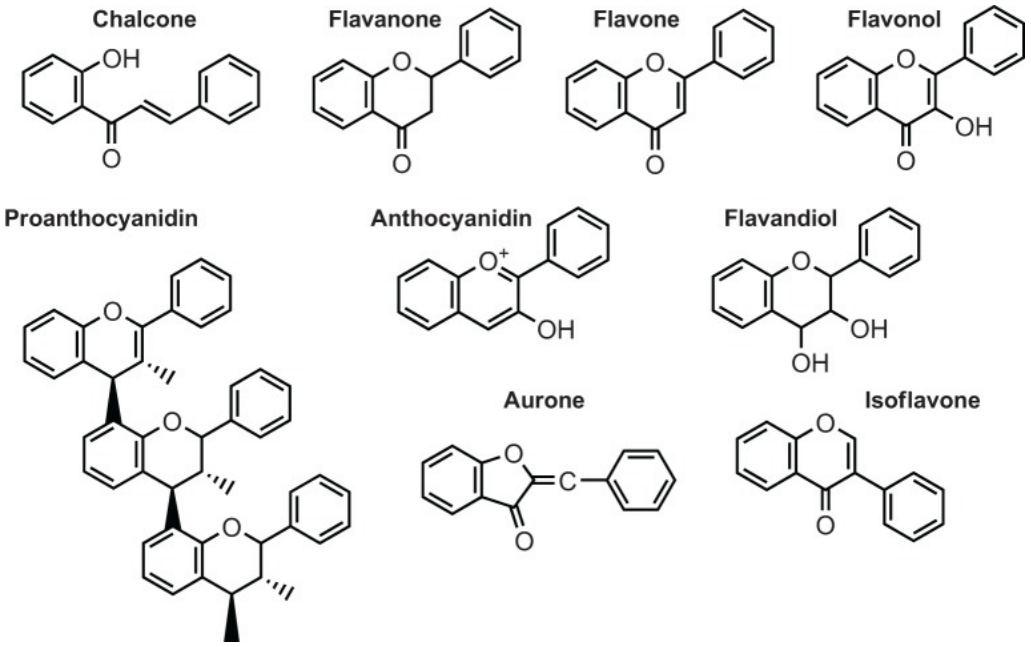


Figure 14: Structure of the different classes of flavonoids **(Falcone Ferreyra and *al*., 2012).**

II.2.2. Alkaloids

The term «alkaloid» was introduced by W. Meisner at the beginning of the 19th century, the admitted definition of alkaloids is that given by Winterstein and Trier in 1910. An alkaloid is an organic nitrogen substance of plant origin that has an alkaline character and a complex heterocyclic molecular structure. **(Badiaga., 2011).**Generally., alkaloids are produced in growing tissues: young leaves., young roots. Then, they go to different places and during these transfers. they can be modified .In many plants, alkaloids are found in the flower parts, fruits or seeds, these substances are found concentrated in the vacuoles.**(Krief., 2003).**These are relatively stable compounds that are stored in plants as products of different biosynthetic pathways.**(Mauro., 2006).**

- **Chemical structure and classification**

Although there is no uniform classification of alkaloids, they can generally be classified into the following major groups: pseudoalkaloids, protoalkaloids and true alkaloids **(Shin and *al*., 2018).**

A- Pseudo-alkaloids

The pseudo-alkaloids do not possess intra-cyclic nitrogen and the incorporation of nitrogen into the structure takes place in the final phase, Precursors of amino non-acids can also

produce pseudo-alkaloids. They can be derived from phenylalanine or acetate. Capsaicin, caffeine, ephedrine are very common examples of pseudo-alkaloids. **(Dey and *al.*, 2020).**

B- Protoalkaloids

This type of alkaloid contains a nitrogen atom. which is derived from an amino acid but is not part of the heterocyclic nucleus system. L-Tryptophan and L-tyrosine are the main precursors of this type of alkaloid. Yohimbine, mescaline and hordenine are the main alkaloids of this type. **(Dey and *al.*, 2020).**

C- True alkaloids

This type of alkaloids is obtained from amino acids and they share a heterocyclic core containing nitrogen. They are highly reactive in nature and have strong biological activity. They form water-soluble salts, and many of them are crystalline in nature, which conjugate with acid and form a salt. Almost all veritable alkaloids have a bitter taste and are solid, except nicotine which is a brown liquid. **(Dey et *al.*, 2020).**Their presence in plants comes in three forms: in the free state, in the form of Noxide, or in the form of salts. Various amino acids such as L-phenylalanine/L-tyrosine, Lornithine, L-histidine, L-lysine are the main sources of veritable l alkaloids **(Dey and *al.*, 2020).**

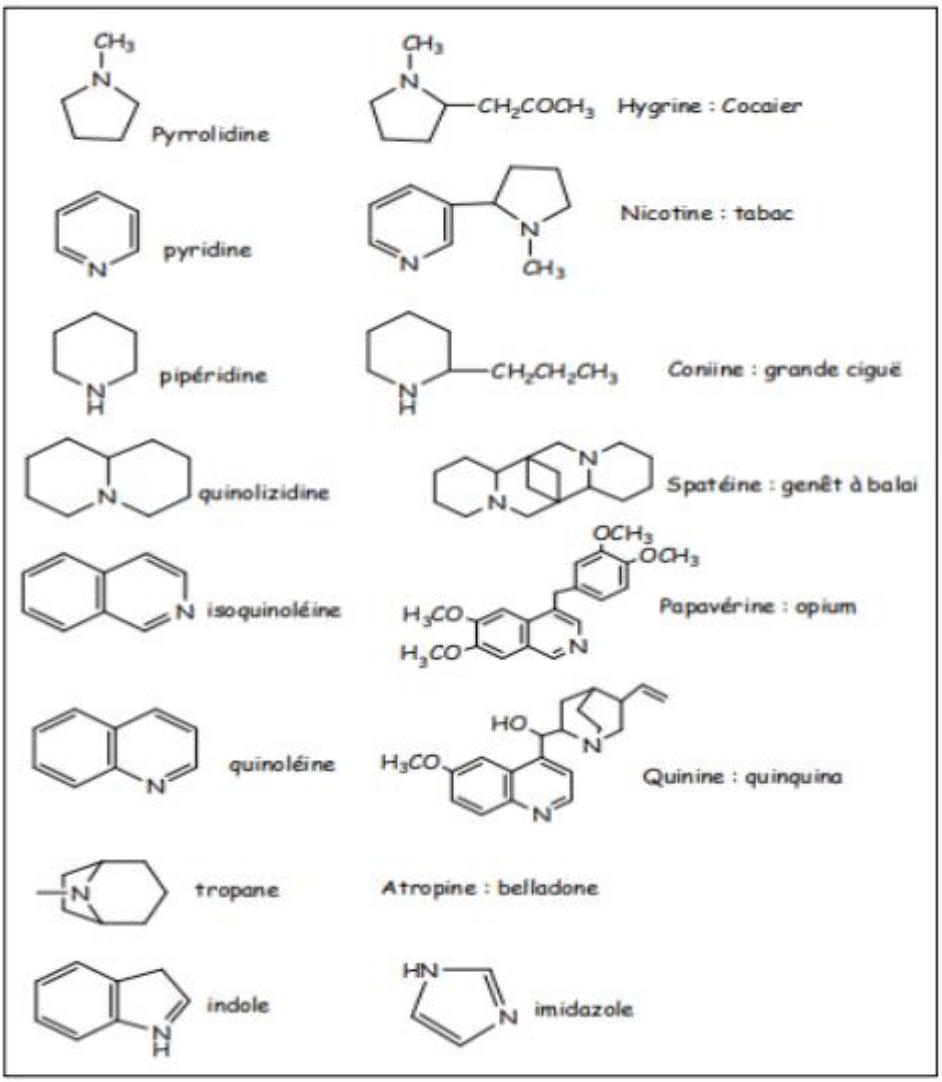


Figure 15: Major structural types of alkaloids **(Yalavarthi., 2013).**

II.2.3. Terpenoids

The term terpene invented by Kekulé, comes from their historical origin of the turpentine tree: «Pistacia terebinthus»**(Ayad., 2008).**The term terpenoid is assigned to all compounds possess a molecular structure constructed of a 5-carbon monomer called isoprene, these compounds are predominantly of plant origin**(Malecky., 2005).** Synthesized by plants, marine organisms, fungi and even animals **(Benaissa., 2011).**The exploitation of these compounds carries out the form of oils extracted from plants (essential oils) by means of distillation**(Malecky., 2005).**

III. *Tribolium Castaneum*

III.1. General Characteristics of *Tribolium castaneum*

Insects of stored product including *Tribolium castaneum* represent a very important part of the pests of stored products **(Syed Shayfur and *al*., 2007).**They can cause significant losses by reducing the quality and quantity of stored products. According to the Food and Agriculture Organization of the United Nations (FAO), losses due to insect pests correspond to 35% of the agricultural production world production **(Anonyme., 2009).**

III.2. Origin and geographical distribution

It is found in all parts of the world. In cold climates, only in high temperature storage **(Aziez and *al*., 2003).**it is a cosmopolitan species, distributed worldwide which according is probably native to India. **(Lepesme., 1944).**

III.3. Taxonomy

Table 05: The classification of this pest according to Perrier, (1961.,1964) and Weidner and Rack (1984)

Taxonomic rank	Nomenclature
Phylum	*Arthropoda*
Class	*Insecta*
Order	*Coleoptera*
Suborder	*Polyphaga*
Family	*Tenebrionidae*
Genus	*Tribolium*
Species	*Tribolium castaneum Herbst*

III.4. Morphological description of the different stages of development

It is an insect belonging to the family Tenebrionidae. The adult is 3 to 4mm tall of uniform reddish brown colour. It is narrow, elongated, with parallel edge, with pronotum almost as wide as the elytra and not previously edged. The last three items in the antennas are significantly larger than the next ones. Unlike *Tribolium castaneum*, the chaperone does not exceed beyond the eye laterally. The larva measures 6mm, about 8 times longer than wide of very pale yellow at maturity, with laterally some short yellow bristles. The head capsule and the dorsal side are slightly reddish **(Camara., 2009).**

A- Eggs

Eggs are ovular, without carving. They are 0.6mm long on average **(Steffan., 1978)**. Eggs hatch after incubation, averaging one week at 25°C. The extreme durations are 4 days at 38°C and 2 weeks at 19°C **(Multon., 1982).**

B- Larvae

The larvae are vermiform, yellow-spotted with a head capsule. The mouthparts and dorsal face are slightly darker **(Lepesme., 1944).**The thoracic segments have 6 well-developed legs and the same colour as the rest of the body . The first pair of legs is more developed than the other two pairs **(Dajoz., 2002)**.The young larvae gradually turn yellow, except for the mouth parts and the darker head. The larvae gradually lose their mobility during the development which consists of 7 to 8 moulting, sometimes much more in adverse conditions, and which lasts six weeks to four months. They live directly in grain products or feed on damaged grains. The Older larvae ascend near the surface before moving on. The sex can be determined at this stage by microscopic observation **(Multon., 1982).**



Figure 16: larva of *T.castneum* **Arab., R. (2018).**

C- Nymph

It is white and bare. The segments of its abdomen are ex laterally in rectangular blades with crenellated edges **(Balachowsky., 1962).** The nymph remains unprotected and is unable to move. The sex may be clearly visible at this stage.



Figure 17: *Tribolium castaneum* nymph **(Kassimi., 2014).**

D- Imago

After having undergone an imaginal molt. The nymph gives an imago. At its emergence. The imago becomes clear. After two to three days, the color turns red brown and reaches 3-4 mm in size **(Balachowsky and *al*., 1936).**

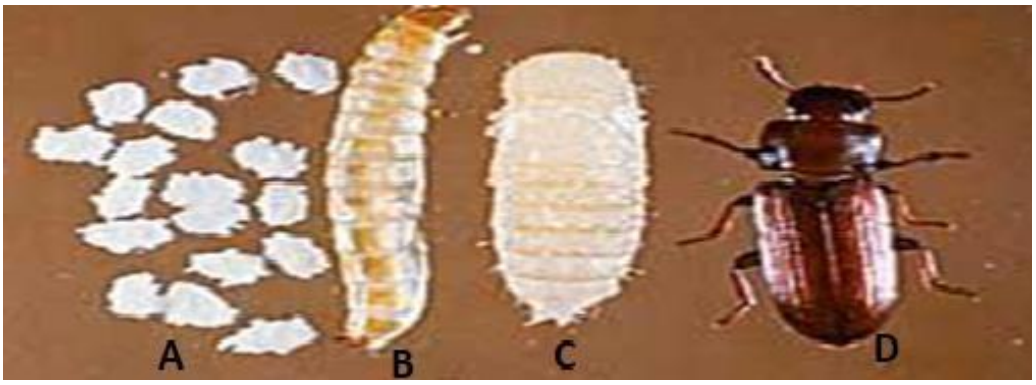


Figure18 :(A)Egg., (B)larva., (C)nymph and (D)Adult of *Triboluim castaneum*. **(Herbst., 1797) (Camara., 2009).**

III.5. Life cycle

The longevity of the insect is 2 to 8 months depending on the abiotic conditions. From the age of three days, the female lays about ten eggs daily, which hatch after five days around 30°C. Eggs are laid loose on seeds and are difficult to detect. The larvae move freely in infested foodstuffs and pupate without a cocoon. At 30°C, the larval life lasts about three weeks and the adult emerges from the pupa six days after its formation. It is a species whose thermal optimum is between 32 and 33 °C, its development ceasing below 22 °C and which resists very well to low hygrometries. The female lays between 500 and 800 eggs. The cycle lasts about one month. The longevity of the insect is 2 to 8 months depending on abiotic conditions **(Delobel and Tran., 1993; Cruz and *al*.., 1988).**

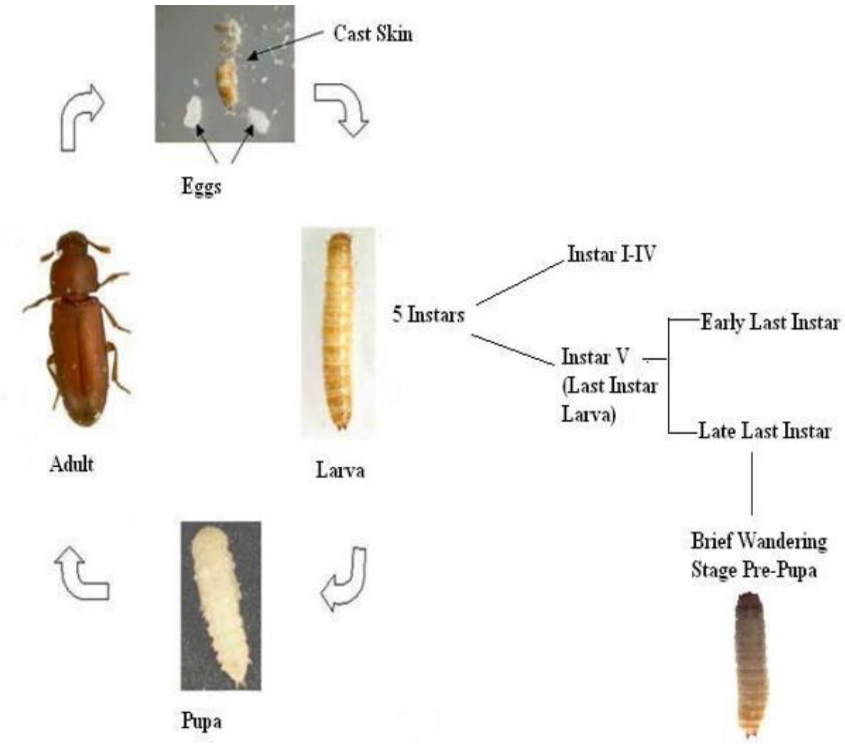


Figure19: Life cycle of *Tribolium castaneum* **(Sreeramoju., P.,2006).**

III.6. Damage caused by *T.castaneum*

Tribolium can cause serious damage by consuming food, but they also contaminate these products with feces, smell corpses and molts. In addition, moisture from the metabolism of their proliferation and nitrogenous excretion products promotes the appearance of molds in storage leagues **(Mebarkia and Guechi., 2001).** *Tribolium* mainly looks for starchy, powdery foods such as flour **(Lepesme., 1944)** Adults secrete a nauseating substance rich in quinones that imparts a particularly unpleasant smell.

On peanut seeds, *Tribolium* causes a notable increase in the content of fatty acids in the oil extracted from them and attacks wheat, corn, barley, sorghum, millet: manioc, yam, peanut, cotton, castor, cocoa **(Delobel and Tran., 1993 Cruz and al., 1988).**



Figure 20: Damage caused by adult *Tribolium castaneum* in flour. (**Nigel Cattlin 2007).**

III.7. Biological control against *T.castaneum*

II.7.1 Use of insecticidal plants

The use of native plants in crop conservation was practised even before the appearance of synthetic insecticides. Plants are used against pests for their repellent, contact or fumigating effects. The active molecules may vary from one family to another and within the same family and the sensitivity may differ for a given insect from one stage to another **(Boeke and al.., 2004).**

IV. *Callosobruchusmaculatus F*

IV.1. General Characteristics of *Bruchinae*

The family *Bruchidae* is divided into two groups, the first includes *bruchids* developing in the fields,in the still green seeds characterizingby a single annual generation (univoltine species) such as *Bruchuspisorum* (pea weevil),*Bruchusruf imanus* (broadbean weevil) or *Bruchuslentis* (lentil weevil).The second group contains *bruchids* that are poured inside the warehouses, the dry seeds, they have another annual generation (polyvoltine species) in this case *Callosobruchus maculatus F., Acanthoscelide sobtectus ., Caryedon serratus* and *Bruchidusa trolineatus* **(Delobel and Tran., 1993).**

IV.2. Origin and geographical distribution

The species *Callosobruchus maculatus* is native to Asia and Africa **(Southgate., 1978);** and subtropical climatic affinity **(Lepesme., 1944 ; Fleurat lessard., 1980).**

This species has become a cosmopolitan pest with the increase of international traffic **(Ridet., 1992)** of legume seeds in many sub-Saharan regions of sub-Saharan regions of West Africa **(Fleurat lessard., 1980 ; Caswell., 1981).**

IV.3. Taxonomy

According to (**Balachowsky., 1962)**, the systematic position of *Callosobruchus maculatus* is as follows:

Table06: The classification of this pest according to **(Balaschowvsky., 1962).**

Taxonomic rank	Nomenclature
Phylum	*Arthropoda*
Subphylum	*Hexapoda*
Class	*Insecta*
Order	*Coleoptera*
Suborder	*Polyphaga*
Family	*Bruchidae*
Genus	*Callosobruchus*
Species	*Callosobruchus maculatus*

II.4. Morphological description of the different stages of development

II.4.1.Eggs

This one has an ovoid shape, it measures 0.4 to 0,7mm long by 0.3 to 0.45mm wide and its color may vary. Indeed, at its deposition it is of translucent color, if the hatching occurs it becomes milky white **(Giga and Smith., 1987).**



Figure 21: Egg of *Callosobruchus maculatus* F. **Karbache., F. (2009).**

II.4.2. Larvae

(Balachowsky., 1962) notes that there are four larval stages.

- The first stage larva is chrysomelian with short robust legs.
- That of the second stage is apode and of rhynchophorian type.
- The third stage larva reaches a length of 4 mm.
- The larva of the fourth and last stage is distinguished by its fleshy body.

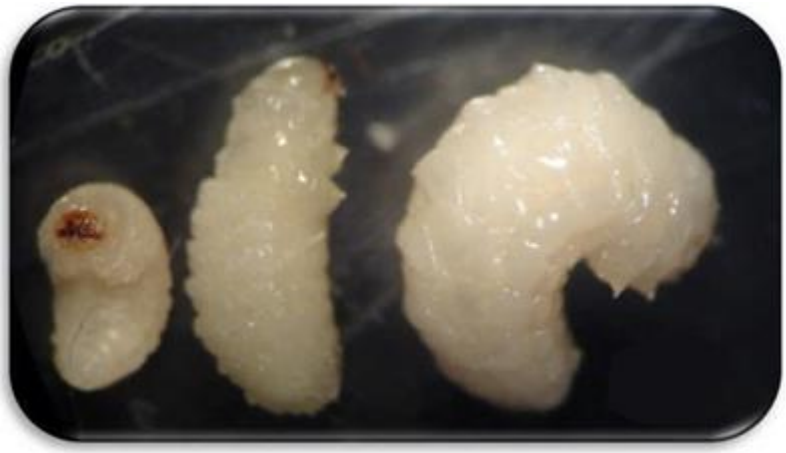


Figure 22: Larvae of *Callosobruchus maculatus* F (**Derradji & Meziani.., 2016).**

II.4.3. Nymph

The pupation takes place in the seed itself or near the tegument. It lasts about 6 days **(Balachowsky., 1962; Mouri., 1999).**

II.4.4. Adult

Balachowsky (1962) states that adults of *C. maculatus* average 2.8 to 3.5 mm of long and it is black and red.

Its elytra are black with brown areas with white and golden pubescence **(Delobel and Tran 1993)**.Males have black antennae with the first four articles red and which are entirely red in females. Adults do not feed; they live on reserves accumulated during development larvae **(Balachowsky 1962).**



Figure23: Adult *C. maculatus* in dorsal view (A) male., (B) female (**Ait Djebbara., 2015).**

II.5. Life cycle

C. maculatus completes its life cycle., from egg to adult., in 28 days under laboratory conditions, 27°C and 70% relative humidity **(Hoffmann., 1945)** Females lay 75 to 100 eggs. This oviposition lasts from 15 days to one month, with an average daily oviposition of 3 to 12 eggs **(Larson and Fischer in Balachowsky., 1962).**

(Kellouche., 2005) reports that the development of the 4 larval stages and that of the pupa takes place entirely inside the grain. To penetrate the seed. the young larva leans on the inside of the chorion and then digs into its gallery. As the larva penetrates, it rejects back of the powder of grain which accumulates under the chorion of the egg which becomes then opaque. According to the same author. The average length of the development cycle (from egg to adult) is 28 ± 3 days in chickpea seeds.

Table 07: Duration (in days) of the different states and larval stages of *C.maculatus* **(KELLOUCHE., 2005)**

States and larval stages	Duration (days)
Embryogenesis	7±1
1st stage larva	2±1
2nd stage larva	2±1
3rd stage larva	6±1
4th stage larva	5±1
Nymphose	6±1
Total duration (days)	28±3

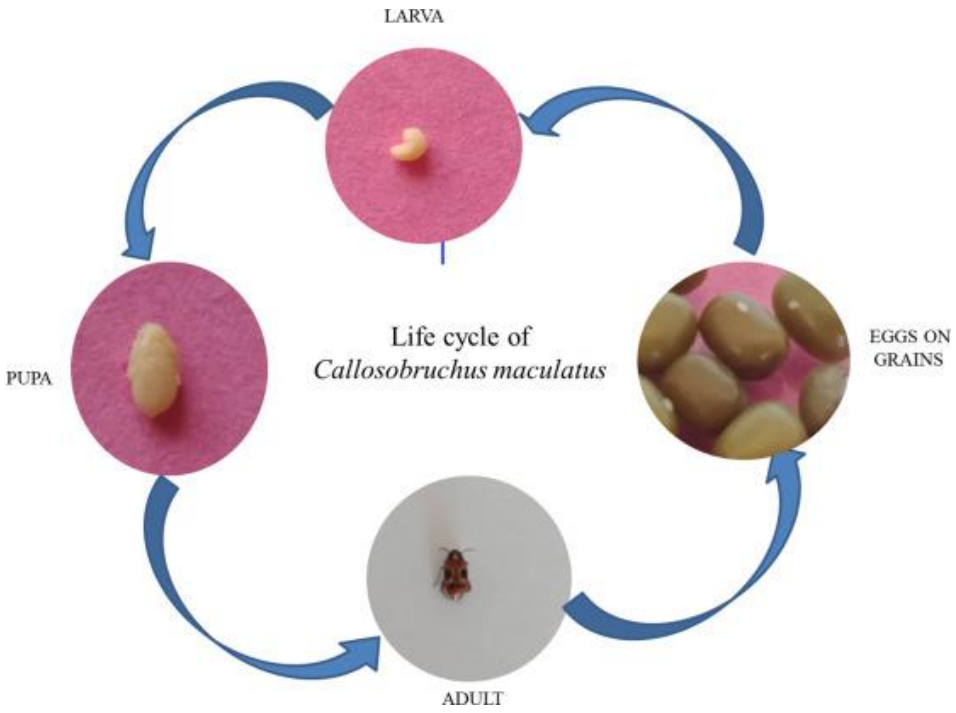


Figure 24: Life cycle of *Callosobruchus maculatus (***Hajam**., **Y. A.., & Kumar., R. 2022).**

II.6. Damage caused by *C.maculatus*

Legume seeds are heavily attacked during storage by *Callosobruchus maculatus* F **(Singh and Emden., 1979., Shih- chieh and *al*., 2002)**.As a result, it affects the mass of seeds., their germinative potential and their market value **(Kumar and *al*. , 1993).**



Figure 25: Damage caused by *C.maculatus* **(Anonymous., 2021).**

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The damage caused by this species *Callosobruchus maculatus* F. is mainly due to the larvae which devour the seeds (**Kellouche., 2005).**



Figure 26: Damage caused by *C.maculatus* **(Anonymous., 2021).**

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II.7. Biological control against *C.maculatus*

Every living organism has natural enemies or diseases that regulate its populations **(Altieri and *al*., 2005; Mahr and *al*., 2011).**It is these natural antagonists of pests that biological control methods bring to bear. Biological control is a viable and acceptable strategy where beneficial biological agents as formulated products are applied to grain infested with stored commodity pests **(Mishra and *al*., 2018)** and potentially active against bruchid populations **(Soudararajan and *al*., 2012)**.

Numerous parasites and predators of *C. maculatus* have been identified, such as Hymenoptera which thrive in granaries at the expense of eggs and larvae of the bruchid. *Dinarmusbasalis* and Eupelmus vuilleti are the most effective **(Sanon and *al*.., 1999).**

Part 2

Experimental study

Chapter II

Materials and Methods

Chapter II: Materials and methods

II. Objective

The present work consists of a bioinsectiside study of plant extract (ethanolic.,aqueous) and AgNPs of leaves, stem and root of *A.Belladonna* as well as the evaluation of its insecticidal activity in vitro. This work is carried out jointly with the Biology laboratory of the Faculty of Sciences., Yahia Fares University. The internship proceeded from April to month of June 2022.

II.1.Material

II.1.1. Biological material

II.1.1.1. Plant material

The plant model was harvested manually from Jijel more precisely in the Taza National Park at an altitude of 550 meters (36° 41′ 05 Nord., 5° 36′ 48 Est.);during March.

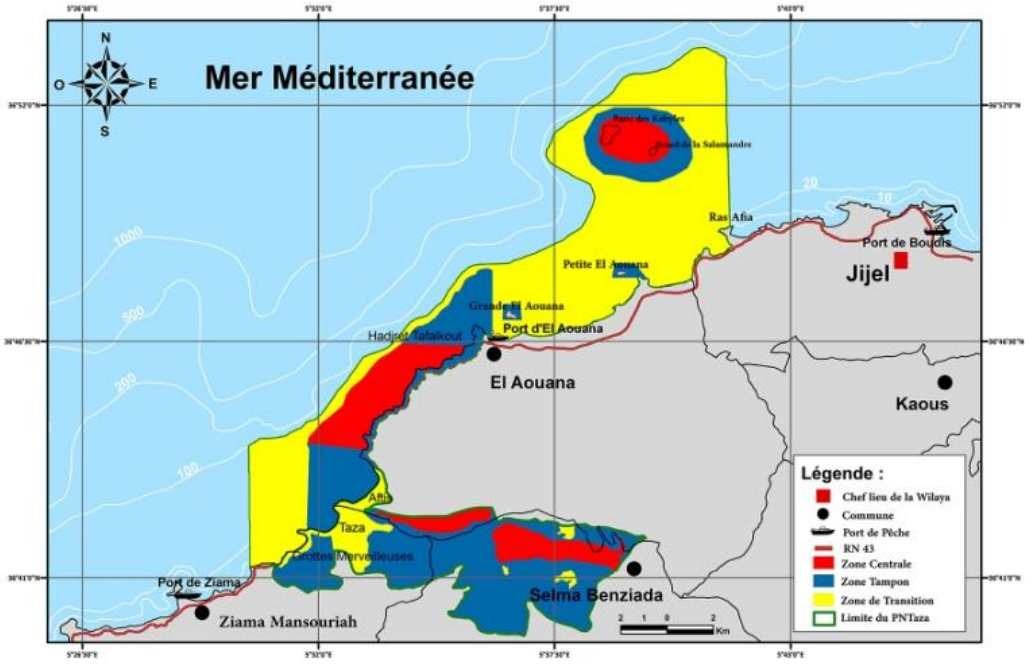


Figure 27: Geographic Maps presents Taza National Park.

Source: (Taza National Park).



Figure 28: *Atropa Belladonna* (Original).

II.1.1.2.Animal material

Two species of insects were chosen as models to study insecticidal activity., *Tribolium confusum* used were isolated from a recovered sample of infected wheat from the cooperative of cereals and dry vegetables of Berrouaghia (CCDV)., and *Callosobruchus maculatus* from stored chickpea., elvage is done at the laboratory Faculty of Science of University Yahia Fares Medea.

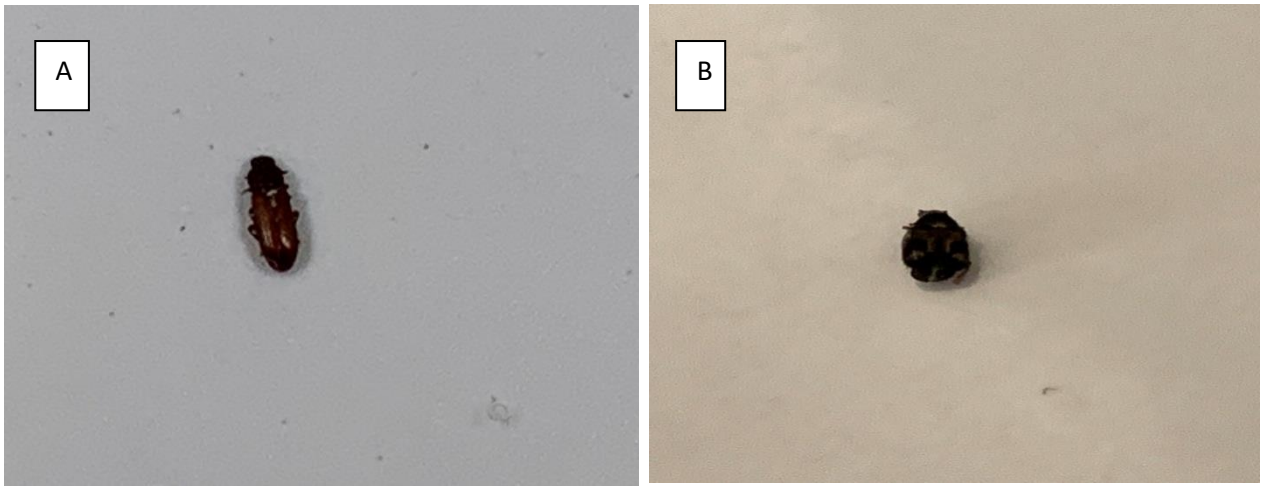


Figure 29: A- *Tribolium confusum.* *B- Callosobruchus maculatus*from (original).

II.2.Methods

II.2.1. Method of drying., grinding and preserving vegetable

II.2.1.1. Method of drying

After harvesting we put the organs on a dry and clean paper., far from light and heat. The organs of *Atropa Belladonna* were completely dried within 25 days.



Figure30: The different organs of *Atropa Beladonna* during the drying process. (Original)

II.2.1.2. Method of grinding and preserving powder

The different organs were ground to a very fine powder by an electric grinder .We have stored the powder in glass boxes., tightly closed., covered with aluminum foil and stored in the dark., away from light and moisture.

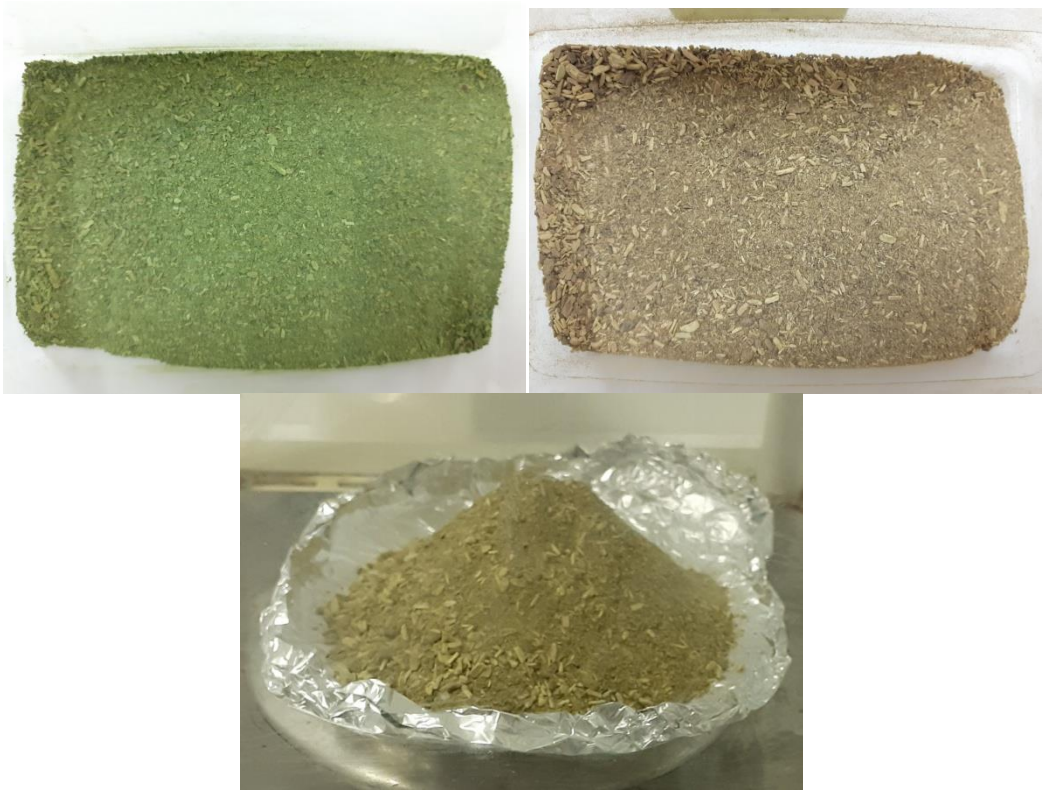


Figure31: Powder of leaves, stem and root of *Atropa Belladonna*. (Original)

II.2.2. Extraction

II.2.2.1. Preparation of Plant extracts

The extracts used in our study prepared by maceration of leaves, stem, roots of the species (*Atropa Belladonna*) are milled to submit to two types of extraction: aqueous and organic (water-ethanol).

A- Aqueous extraction (maceration with distilled water)

According to the protocol of **SANOGO and al (2006)** with some modifications, the extraction was performed according to the following steps:

✓Maceration of 10g of powdered plant material in 100 ml of water.

✓Magnetic stirring at room temperature for 24 hours.

✓After, the macerate subjected to a double filtration on cotton hydrophilic then on filter paper whatman n°1.

✓The aqueous extracts are then dried in the oven (45C) for 7 days.

✓The dry extracts obtained are rubbed and kept at 4° C until use.

B- Hydro-alcoholic extraction

According to **Mugiraneza JP (2009)** with some modifications, the extraction was carried out according to the following steps:

✓ Maceration of 10g of plant powder in 100ml 70% hydro-ethanolic solution (ethanol/water, 70/30, v/v);

✓Magnetic stirring at room temperature for 24 hours.

✓After, thehydro-alcoholic macerate subjected to a double filtration on cotton hydrophilic then on filter paper whatman n°1.

✓Thehydro-alcoholic extracts are then dried in the oven (45C) for 7 days.

✓ The dry extracts obtained are rubbed and kept at 4° C until use.

II.2.3. Silver nano-particule

II.2.3.1. Preparation of the aqueous extract

According to the protocol of (**Gomathi., 2017)** with some modifications., the extraction was performed according to the following steps:

✓Maceration of 10g of powdered plant material in 100 ml of water.

✓Magnetic stirring at 60C° for 10 minutes.

✓After., the macerate subjected to a double filtration on cotton hydrophilic then on filter paper whatman n°1.

✓The extract broth was stored in refrigerator at 4 °C for the further use.

II.2.3.2. Biosynthesis of silver nanoparticles

According to the protocol of **(Rajput., 2020)**with some modifications., the extraction was performed according to the following steps:

✓10 mL of freshly prepared aqueous Leaves extract was added to 90 mL of 1 mM aqueous silver nitrate.

✓The reaction mixture was heated at 60±2 °C for 30 min with continuous stirring

✓The solution containing nanoparticles was centrifuged 4 times at 6000 rpm for 30 min to separate the AgNPs., The pellet was washed with distilled water severaltimes to remove impurities and 90% ethanol to obtain pure NPs Ag powder.

II.2.3.3 Preparation of AgNPs dilutions

The preparations of the dilutions were obtained according to the following protocol :

100%	0.5 ml of AgNPs suspension + 0.5 ml of distilled water
75%	0.5 ml of AgNPs suspension + 1 ml of distilled water
50%	0.5 ml of AgNP suspension + 1.5 ml of distilled water
25%	0.5 ml of AgNPs suspension + 2 ml of distilled water

II.2.4. Evaluation of biological activities

II.2.4.1. Evaluation of insecticidal activity

A- Doses Preparation

During our experiment, we tested four doses of aqueous and ethanolic extracts of three organs (leaves: stem: root) of *Atropa Belladonna* against adults of *Tribolium* and *Bruchus* by the contact test.

The three doses were prepared by dissolving the plant extract in 1 ml of distilled water.

B- Application

For the application. The batch (T) is composed of 3 petri dishes. In each one a filter paper is placed. Each petri dish, we introduce 10 Tribolium. So 30 individuals in the batch (T).The process is the same for the other four batches D1, D2, D3 and D4 treated at 0.2mg, 0.5mg, 1mg and 2mg respectively.

Finally, the petri dishes are covered. We did the counting after 2 hours, 4 hours, 8 hours, 12 hours and 24 hours. The same process for the adults of the *Bruches*.

C- Calculation of DL 50 and TL 50

1- Mortality rate

2- Lethal Dose 50: LD_{50}

3- Lethal time 50: TL_{50}

1- Mortality rate

Mortality is the first criterion for judging the effectiveness of a chemical or biological treatment. The percentage of mortality observed in control and plant extract treated adults is estimated by applying the following formula **(Ould Elhadj and *al*., 2006)**:

Observed mortality = [Number of deaths/ Total number of individuals] x 100

2- LD_{50}

The lethal dose 50 (lethal dose for 50% of the individuals) is a value which informs us on the importance of the toxic effect of our extract in time. it is an indicator of the toxicity of a substance. To estimate it is necessary:

Calculate the decimal logarithm of the different doses used. **(Annex 02).**

Trace the regression lines: Probits = (log dose)., the equations of the regression lines are of type:

Y = aX+ b Shall be determined. Or:

Y: Adjusted Mortality Probit

X: decimal logarithm of the concentration

a: bridge

3- LT_{50}

To estimate the LT50 (lethal time after which 50% mortality is obtained). the regression lines Probits = (log time) are traced and the equations of the regression lines are then established From these. The LD50 values are determined. **(Annex 03).**

Chapter III

Results and Discussion

III. Studies of insecticidal activities

In this part of the work we evaluated the insecticidal effect of *Atropa Belladonna* extract on the longevity of *Triboliums confusums* and *Callosobruchus maculatus*.

III.1.Evaluation of toxicity

III.1.1.Evaluation of the toxicity of *Atropa Belladonna* extracts on *T.castaneumnou*

According to Figure 32 which illustrates the effects of ethanolic extracts of *A Belladonna* on the longevity of *T.castaneum*note that extract of the Root (RoAq) and the leaves (LeaAq), at dose (D3), have an important effect on the longevity of *T.castaneumnou* which can reach 100% after 12h.

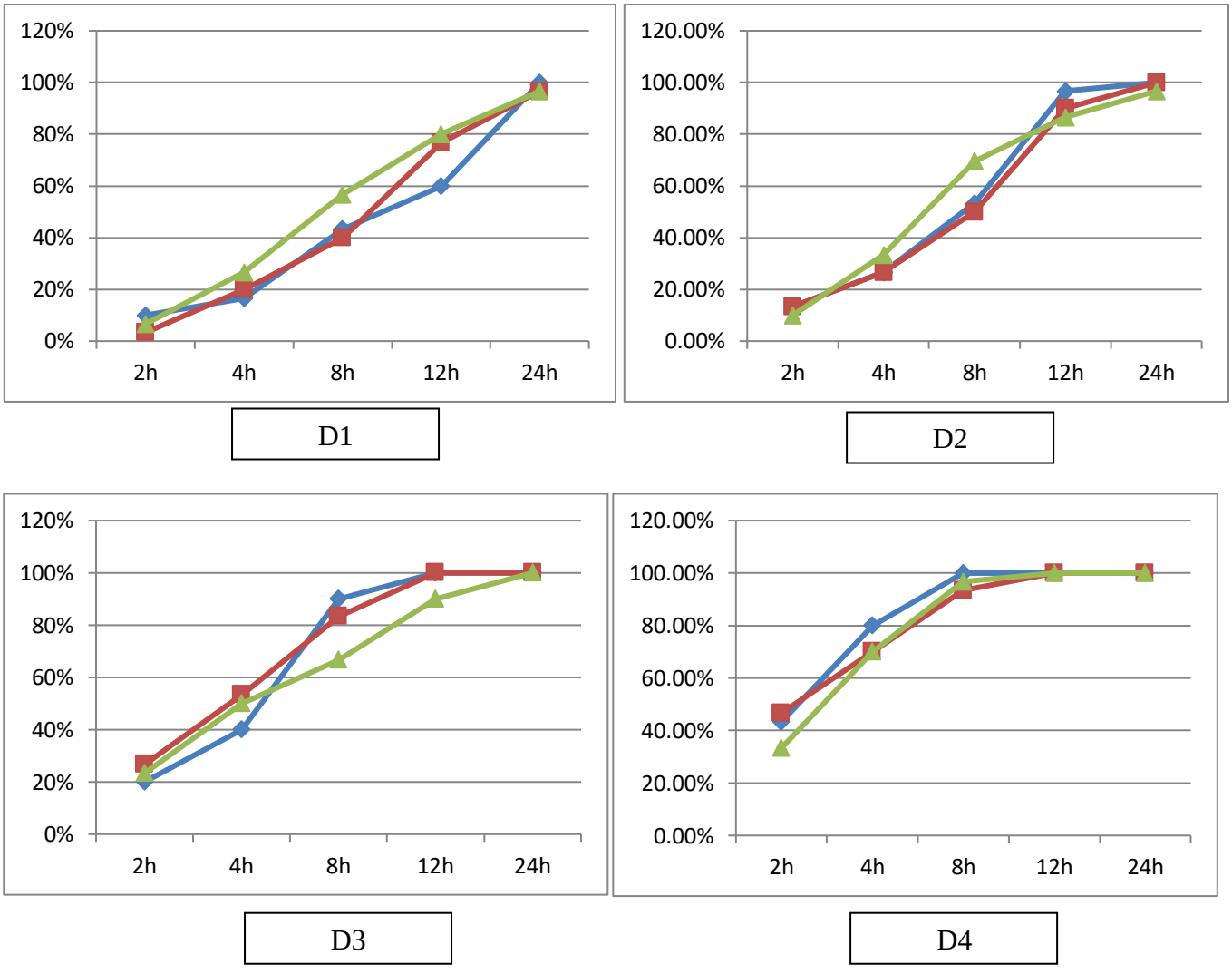


Figure 32: Evolution of the mortality of *T.castaneum* over time as a function of doses of ethanolic extract of *Atropa Belladonna*.

Similarly for the aqueous extracts illustrated by figure 33 , shows the extract of the Root (RoAq) and the leaves (LeaAq) has a mortality rate of 100% at high dose (D4) after 8h.

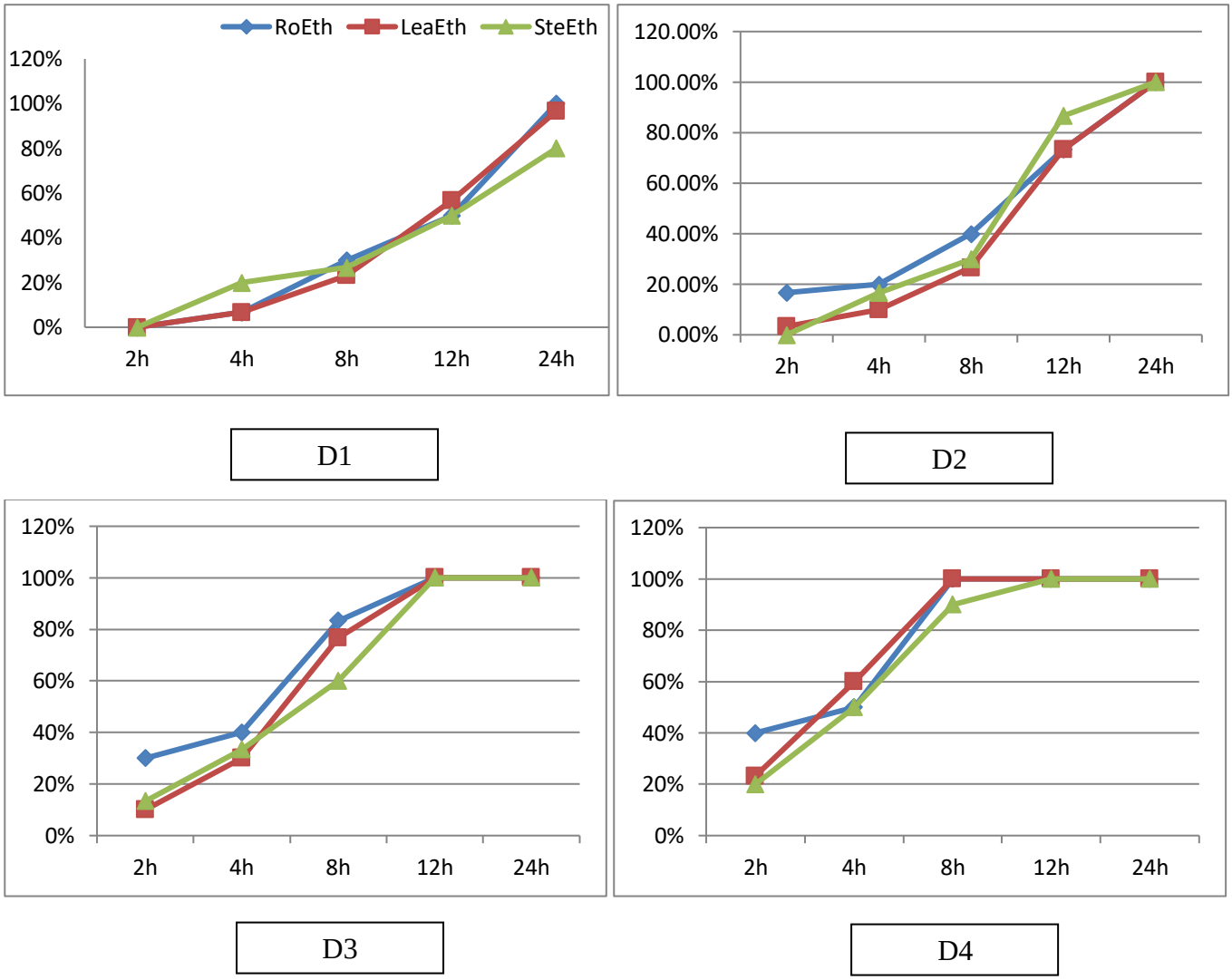


Figure 33: Evolution of the mortality of *T.castaneum* over time as a function of doses of aqueous extract of *A.Belladonna.*

III.1.2. Evaluation of the toxicity of *Atropa Belladonna* extracts on *C.maculatus*

The results of the evaluation of the effects of the different extracts of *A.Belladonna* tested on *C.maculatus* by contact show an effectiveness of these extracts.

According to figure 34 which illustrates the effects of ethanolic extracts of *A.Belladonna* on the longevity of *C.maculatus*, we notice that the root extracts (Ro EtOH) can reach 100% mortality

after 12h with the doses (D3 and D4)., and after 24h with (D1 and D2). Ethanolic extracts of leaves (Lea EtOH) and Stem (Ste EtOH) have a 100% mortality rate after 24 hours with D3.

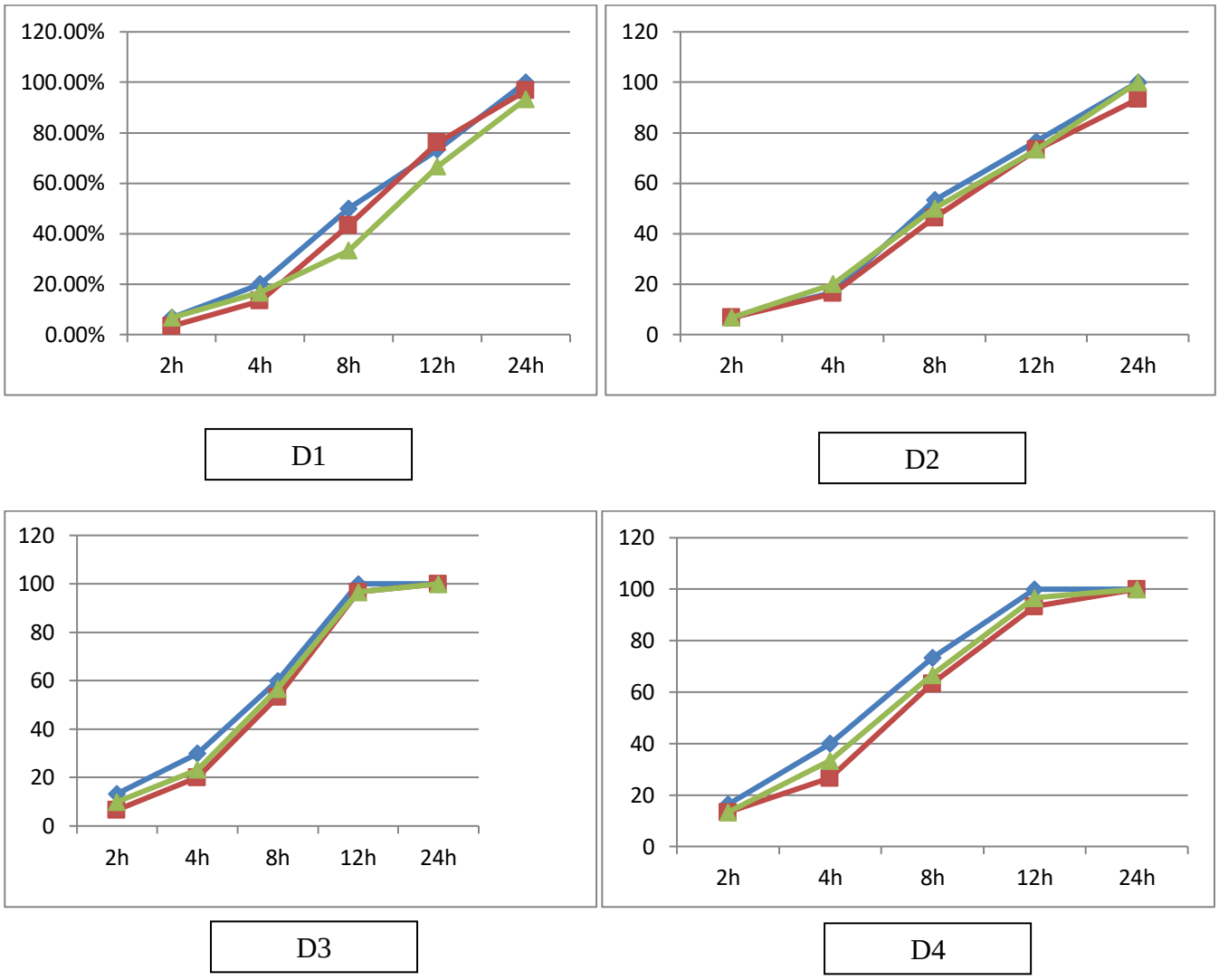


Figure 34: Evolution of the mortality of *C.maculatus* over time as a function of doses of ethanolic extract of *A.Belladonna*.

For the aqueous extracts of *A.Belladonna* shown in figure 35 a 100% mortality rate is reached after 12 hours for the root extract (RoAq) at D4.

For the leaves (LeaAq) and stem (SteAq) a 100% mortality rate is observed after 24 hours at D3.

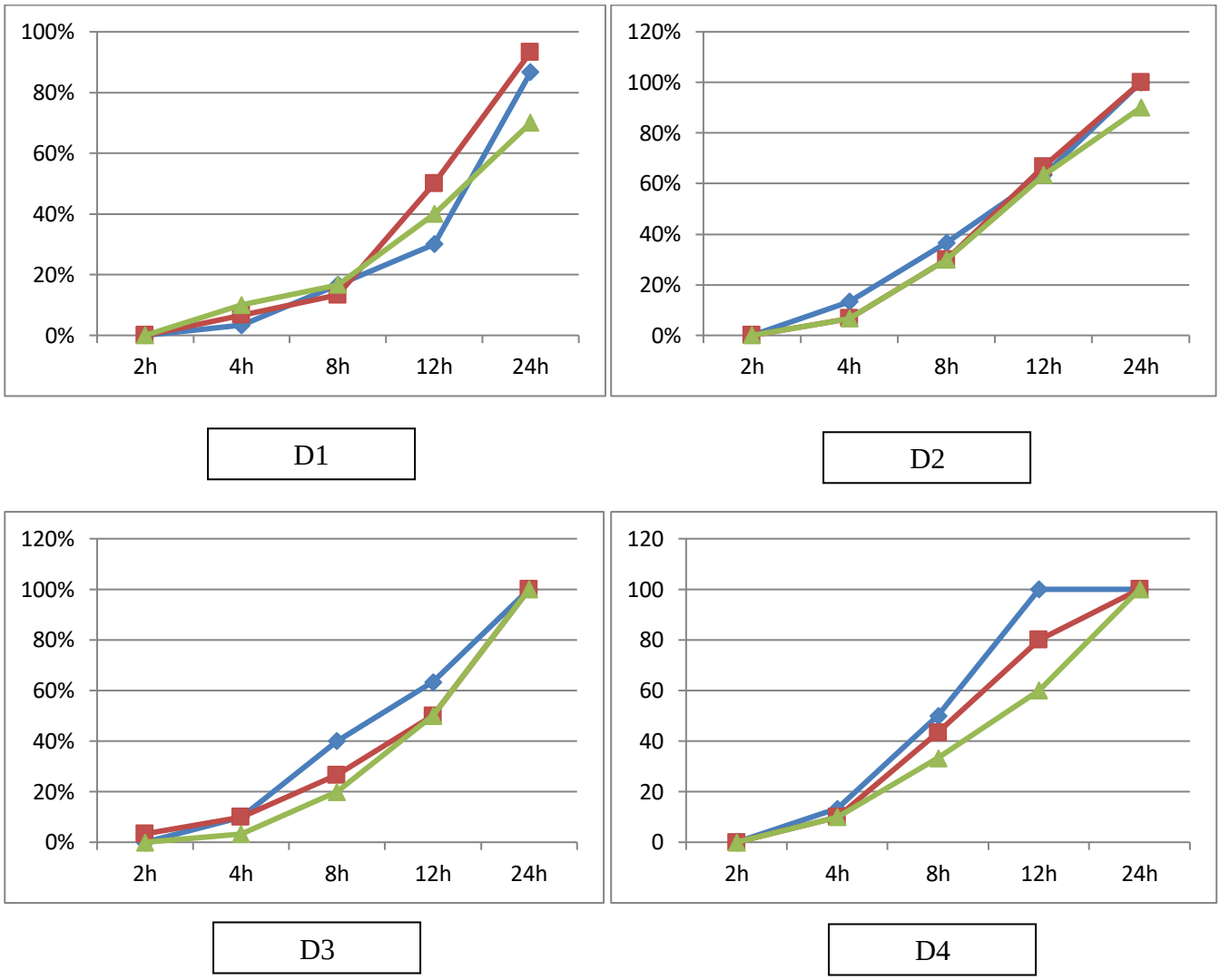


Figure 35: Evolution of the mortality of *C.maculatus* over time as a function of doses of aqueous extract of *A.Belladonna.*

All the results obtained show that the longevity of *T.castaneum* and *C.maculatus* are proportional to the doses of the tested extracts and the duration of exposure.

III.2. Estimation of LD_{50} and LT_{50}

III.2.1. Lethal Doses LD_{50}

The overall analysis of the LD results shows that the effect induced on the survival of C. *C.maculatus* and *T.castaneum* varies considerably between the tested extracts of the species (*A.Belladonna*).

III.2.2.Lethal doses of *A.Belladonna* on *T.castaneum*

From the table below, we noted that the lowest concentrations that cause 50% mortality of *T. castaneum* adults are 111.89 and 153.67 mg/ml for the aqueous extract of red fruits and ethanolic extract of peduncles respectively.

Table 08: Effect of tested doses of different extracts of *A. Beladonna* on the mortality rate of *Tribolium Castaneum* by contact.

Organ	Extract	DL_{50} (mg/ml)
Root	EtOH	0.31
	Aqueous	0.39
	EtOH	0.14
	Aqueous	0.22
	EtOH	
	Aqueous	
Leaves	EtOH	0.33
	Aqueous	0.46
	EtOH	0.2
	Aqueous	0.2
	EtOH	0.04
	Aqueous	0.04
Stem	EtOH	0.20
	Aqueous	0.59
	EtOH	0.11
	Aqueous	0.19
	EtOH	0.20
	Aqueous	0.22

III.2.3. Lethal doses LD_{50} of *A.Belladonna* on *C.maculatus*

According to the table below, the concentrations of the extracts that cause the mortality of 50% of adults of *C.maculatus* are 5.59mg/ml and 57.54 mg/ml respectively for ethanolic extracts of Stem and leaves.

Table 09: Effect of tested doses of different extracts of *A. Belladonna* on the mortality rate of *C.maculatus* by contact.

Organ	Extract	DL_{50} (mg/ml)
Root	EtOH	0.26
	Aqueous	1.76
	EtOH	0.15
	Aqueous	0.42
	EtOH	
	Aqueous	0.72
Leaves	EtOH	0.6
	Aqueous	3.63
	EtOH	7.39
	Aqueous	0.73
	EtOH	0.25
	Aqueous	0.12
Stem	EtOH	0.56
	Aqueous	31.53
	EtOH	0.12
	Aqueous	0.4
	EtOH	0.12
	Aqueous	0.13

III.2.4. Lethal time TL_{50} of *A.Belladonna* extracts on *T.castaneum*

The extracts of the different organs of *A. Belladonna* showed very important insecticidal effects against *T. castaneum*.

The most active extracts are ethanolic extracts of the root at D3 after 1.85 h and the leaves at D4 after 2.33h., followed by aqueous extract of the root at D4 after 2.35h.

Table 10: Lethal time of different extracts of *A.Belladonna* : probities as a function of log time of *Tribolium Castaneum* by contact.

Organ	Extract	Doses	TL_{50} (h)
Root	EtOH	D1	6.39
		D2	4.97
		D3	3.53
		D4	1.85
	Aqueous	D1	9.33
		D2	5.79
		D3	3.36
		D4	2.35
Leaves	EtOH	D1	7.65
		D2	5.23
		D3	3.26
		D4	2.33
	Aqueous	D1	10.92
		D2	25.26
		D3	4.27
		D4	2.62
Stem	EtOH	D1	6.67
		D2	5.65
		D3	4.09
		D4	2.57
	Aqueous	D1	12.15
		D2	8.32
		D3	4.28
		D4	3.66

III.2.5. Lethal time TL_{50} of *A.Belladonna* extracts on *C.maculatus*

The TL_{50} results of *C.maculatus* treated with *A. Belladonna* extracts show that the most toxic extracts are the ethanolic and aqueous extracts of root at D3 and D4 with TL_{50} of 4.33h and 3.90h respectively followed by the ethanolic extract of Leaves at D4 after 4.48h by contact.

Table 11: Lethal time of different extracts of *A.Belladonna* : probities as a function of log time of *C.maculatus* by contact.

Organ	Extract	Doses	TL_{50} (h)
Root	EtOH	D1	6.14
		D2	6.14
		D3	4.33
		D4	3.90
	Aqueous	D1	13.63
		D2	8.78
		D3	8.30
		D4	5.88
Leaves	EtOH	D1	7.87
		D2	7.79
		D3	5.48
		D4	4.48
	Aqueous	D1	14.26
		D2	11.22
		D3	9.71
		D4	8.97
Stem	EtOH	D1	8.54
		D2	6.14
		D3	5.13
		D4	4.65
	Aqueous	D1	11.93
		D2	9.04
		D3	17.01
		D4	8.41

III.3. Evaluation of the toxicity of AgNPs on *T.castaneumnou*

According to Figure 36 which illustrates the effects of AgNPs on the longevity of T.*castaneumnou* note that D1have an important effect on the longevity of *T.castaneumnou* which can reach 100% after 4h.

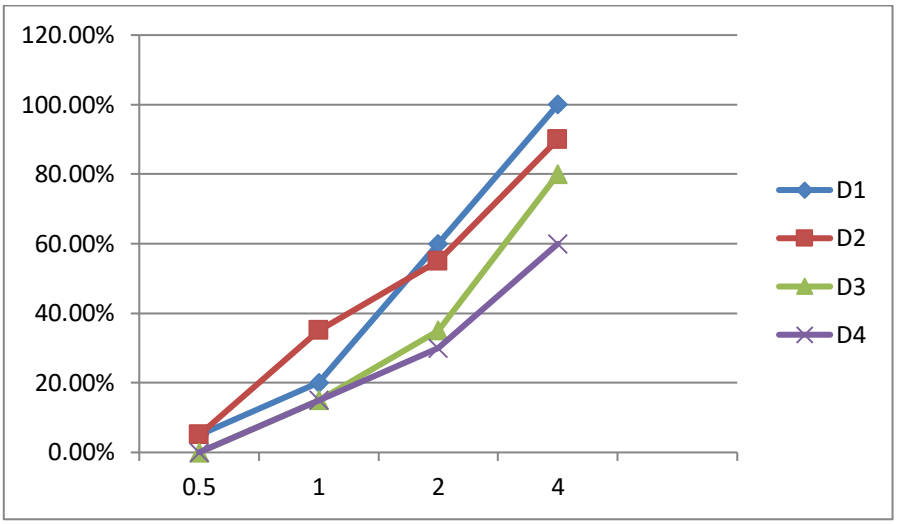


Figure 36: Evolution of the mortality of *T.castaneum* over time as a function of doses of AgNPs of *A.Belladonna*

III.4. Estimation of LD_{50} and LT_{50}

III.4.1. Lethal Doses LD_{50}

The overall analysis of the LD results shows that the effect induced on the survival of *T.castaneum* varies considerably between the AgNPs doses.

III.4.1.1. Calculation of LD50 of AgNPs on *T. castaneum* adults

The results of the efficacy of AgNPs treatment on T. castaneum adults are shown in Table 12 and illustrated in Figure 37, which shows that the treatment LD_{50} is 32%.

Table 12: Adult mortality rates T. castaneum under the effect of AgNPS on adults after 2h of exposure.

Dose (%)	Log (dose)	Mortality rate (%)	Mortality Probit
100	2	60	5.25
75	1.875061	55	5.13
50	1.69897	35	4.61
25	1.39794	30	4.48

III.5. Lethal time TL_{50} of AgNPs on *T.castaneum*

AgNPs showed very important insecticidal effects against *T. castaneum.*The most active Concentrations are Dose 1 (100%) after 1.34 h and and Dose 2 (75%) after 1.54 h.

Table 13: Lethal time of different AgNPs concentrations: probities as a function of log time of *T. castaneum*by contact.

Doses	**Linear regression equation**		**TL_{50} (h)**
D1	Y = 4.,909x - 0.,558	R^2=0.841	1.34
D2	Y= 3.082x + 1.298	R^2=0.978	1.58
D3	y = 6.035x - 3.341	R^2=0.863	2.3
D4	y = 5.404x - 2.795	R^2=0.804	2.75

Discussion

According to the insecticidal activity evaluation, *Atropa Belladonna* extracts were found to be effective against *Callosobruchus maculatus* and *Tribolium castaneum* by contact effect.

Our results are similar to those obtained by several studies that have shown that the use of ethanolic and aqueous extracts of medicinal plants as bio-pesticides reduces the losses caused by insect pests of stored seeds.

In the same context., the work of **Abbasipour and *al..,* (2011)** shows that The acetone Leaves extract of *D. stramonium* has an insecticidal effect on adults of *Callosobruchus maculatus*, also reported that the seed and leaves extract of *D. stramonium* also used to control of *Tribolium castaneum*.

From other works evaluating the insecticidal effects of plants, organic solvent extracts of *Afrostyrax lepidophilus, Trichilia gilgiana, Drypetes gossweileri* and *Zanha golungensis* towards *Sitophilus zeamaïs, Tribolium castaneum* and *Rhyzopertha dominica* is shown by the work of **Toumnou., (2013)**.

Zitouni and Arouche., (2018) tested the effectiveness of thyme extracts and stramoine against *Tuta absoluta*. Biological tests showed that the extract of *D. stramonium* showed a remarkable mortality rate of over 85% and 58% for *T. vulgaris L*. Three days after treatment.

Godara and *al*. (2014) tested the insecticidal effect of the ehanolic extract of *A.Belladonna* on *Rhipicephalus* (Boophilus) microplus. The extract caused a significant reduction ($P< 0.05$) in egg mass production at 10% concentration. In larval packet test, the extract was lethal to larvae in the concentrations of 10% and 20% after 24 h., with LC50 and LC95 values of 1.321% and 4.935%, respectively. Scopolamine and atropine showed 93.3% and 60.0% mortality of adult ticks, respectively but they caused complete (100%) blocking of hatching as well as 100% larval mortality.

These insecticidal activities can be attributed to the different biochemical substances contained in the extracts.

Atropa belladonna is a rich source of flavonoids., coumarin., atropa-amine., scopolamine., and hyoscyamine present in the different organs of *A.Belladonna* **(M. R. Lee., 2007; P. Rita., 2011).**

The work of **(Regnault-Roger and *al* ,.2008),** which shows that ubiquitous polyphenols in nature cause a disruption of the natural motricity of the insect.

Furthermore tannins have a direct toxic effect on some insect species (**Raymond and *al*., 2011).** Tannins influence the growth development and fecundity of several insect pests **(Vandenborre and *al*., 2011).**

Alkaloids have repellent or anti-appetent properties towards insect pests **(Pelletier.,2001).**

Flavonoids also have adverse effects on insects they significantly reduce egg laying in C. chinensis and have toxicity towards adults **(Salunke and *al*., 2005).**

Also., the effect of these compounds can be anti-palatant or toxic., as for example in the caterpillars of Utetheisa ornatrix **(Gronquit and *al*., 2001).** In vitro certain flavonoids inhibit aldose reductase which is responsible for the conversion of glucose into fructose; the C150 has been estimated at 0.0005 mg/ml in the case of quercitrin **(Lee., 2002).**

Conclusion

Conclusion

This research work has made it possible to make a contribution to the study of the biology of Tribolium castaneum and *Callosobruchus maculatus* provides a set of elements that could serve as support for finding alternatives to the control by chemical insecticides against these pests of stored food stocks.

The results of the experiments carried out on the ethanloic and aqueous extracts of the medicinal plant *Atropa Belladonna*, which we tested by contact effect, all seem to have an insecticidal effect on adults of T. *castaneum* and *C.maculatus.*

The values obtained show that the insecticidal activity of the extracts of Atropa *Belladonna* varies in relation to the dose used, where mortality percentages ranging from 50% to 100% were recorded for 12 h in the contact effect test for *T. castaneum* and a percentage ranging from 30% to 96.66% for *C.maculatus*. The efficacy of these extracts on T. castaneum is evaluated by lethal doses. Indeed the LD_{50} obtained confirms that these extracts have insecticidal activities or at doses of 0.14 mg / ml for ethanolic root extract and 0.2 mg / ml respectively for the aqueous and ethanolic Leaves extract with which we obtained the death of 50% of the insect population. The LD_{50} results obtained for *C.maculatus* show that these extracts have insecticidal activities or at doses of 0.6 mg /ml for the ethanolic Leaves extract and 0.72 mg/ml for the aqueous root extract.

The examination of LT_{50} shows that the adults of *T. castaneum* treated with the different extracts of *Atropa belladonna* have LT_{50} vary as a function of time. The highest TL_{50} is reached after 1.85 h for the ethanolic root extract. The highest TL_{50} for adults of *C.maculatus* is reached after 3.9 h for ethanolic root extract.

All the results obtained during this work could constitute alternative or complementary solutions to the use of synthetic organic pesticides for the protection of stored grains. The development of bio-insecticides extracted from plants and the selection of cultivars resistant to this pest is part of the framework of agriculture and sustainable development. Algeria has an abundant and diverse flora that is likely to provide new sources of compounds of plant origin with plant protection properties. Many research perspectives can be drawn from this work, in particular. The extraction of extracts from medicinal plants and the identification of their active ingredients. It would also be interesting to evaluate the insecticidal activity of the major compounds of the extracts on *T. castaneum*and *C.maculatus* and their synergistic or antagonistic effects.

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Annexes

Annex01: The Equipment used



Fridge



Laboratory oven



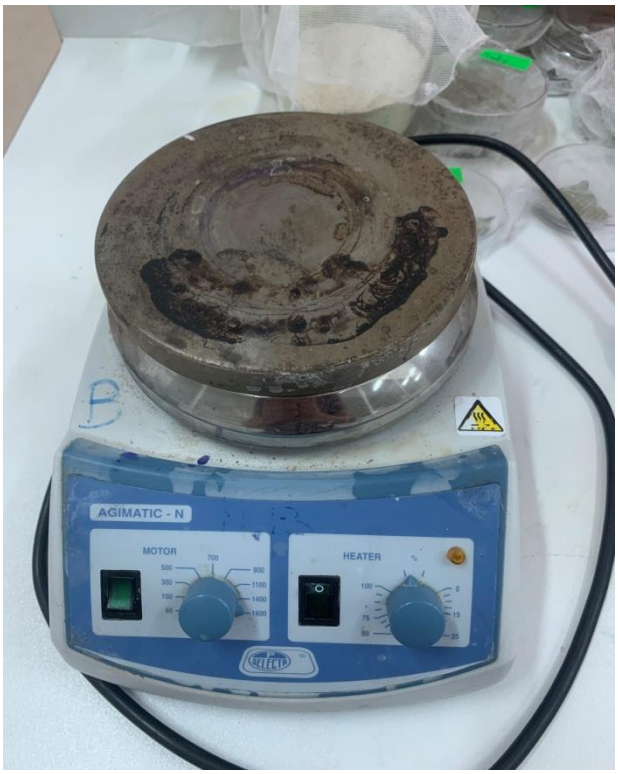
Binocular magnifier



Precisions scale



Vortex



Magnetic agitator



Centrifuge

Annex 02: Lethal Dose (LD_{50}) Estimation

Tracing the regression line of the probits corresponding to the percentages of mortalities corrected as a function of Log doses used made it possible to calculate the LD for the ethanolic extract and the aqueous extract of Atropa Belladonna used by contact.

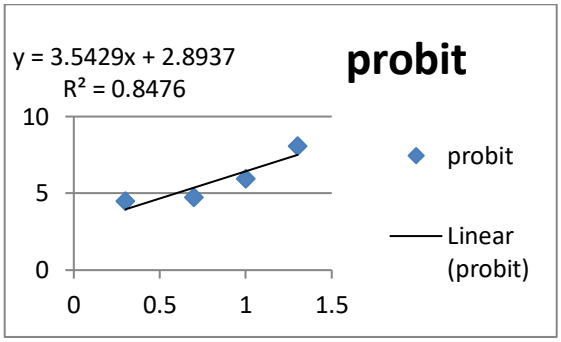


Figure 38: regression line: probits as function of the log doses at the Root (Ro Aq) for 8 h.

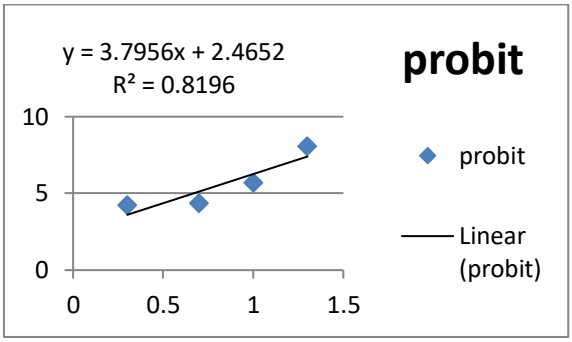


Figure39: regression line: probits as a function Of the log doses at the Root (Ro Aq) for 8 h.

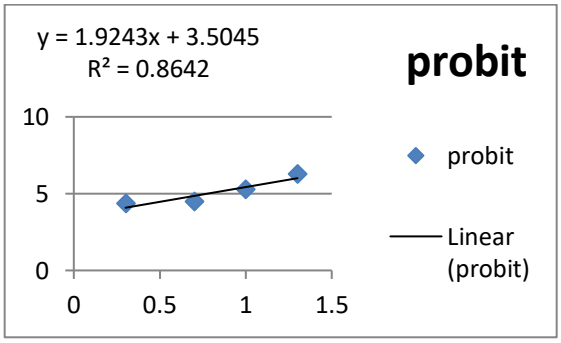


Figure 40: regression line: probits as a functionof the log doses at the Stem (Ste Aq) for 8 h.

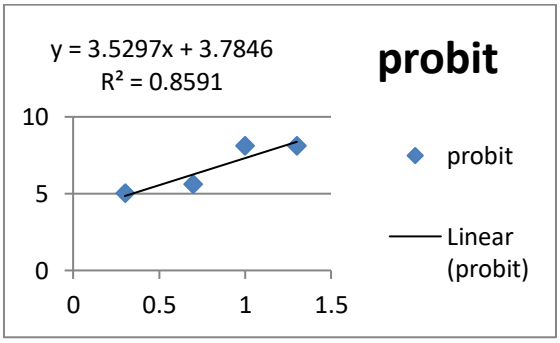


Figure 41: regression line: probits as function Of the log doses at the Root (Ro Aq) for 12h

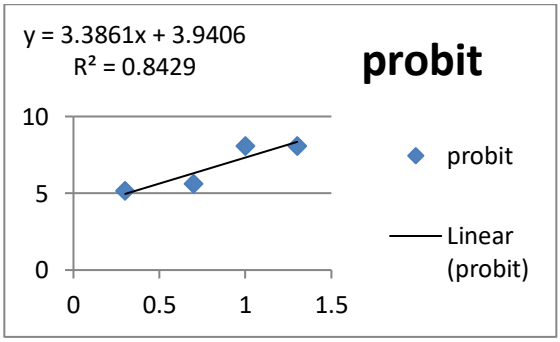


Figure 42: regression line: probits as function of the log doses at the Leaves (Lea Aq) for 12h

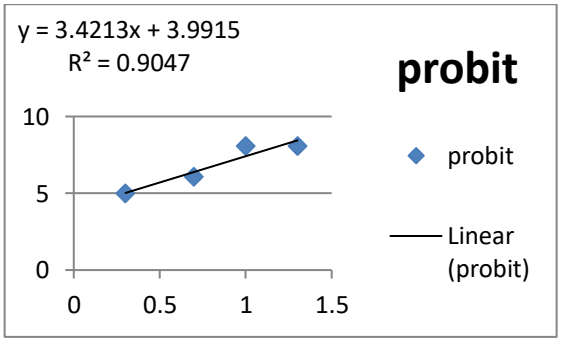


Figure 43: regression line: probits as function of the log doses at the Stem (Ste Aq) for 12h

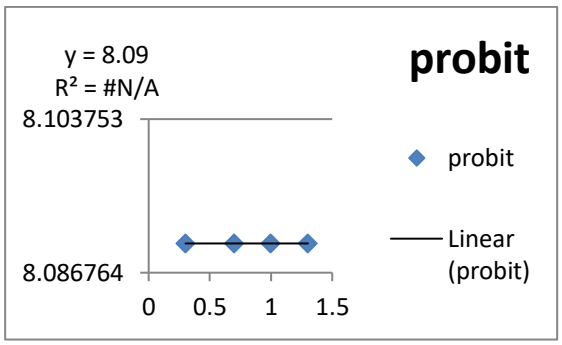


Figure 44: regression line: probits as afunction of the log doses at the Root (Ro Aq) for 24h

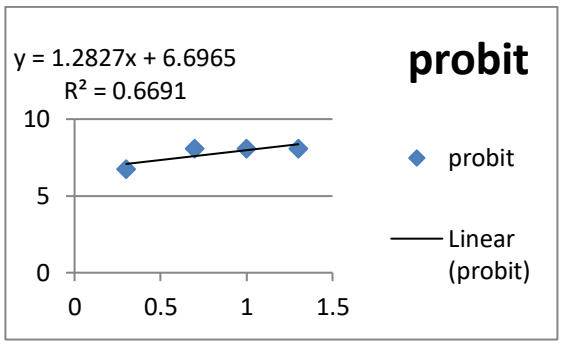


Figure 45: regression line: probits as a function of the log doses at the Leaves (Lea Aq) for 24h

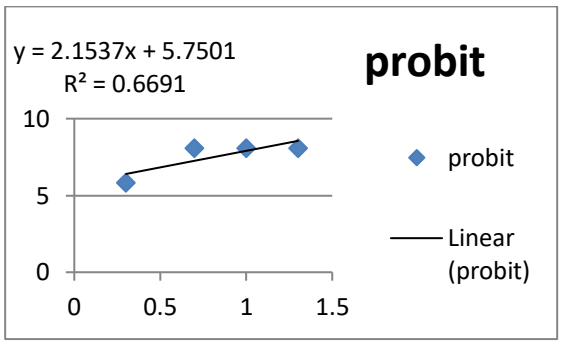


Figure 46: regression line: probits as a functionof the log doses at the Stem (Ste Aq)

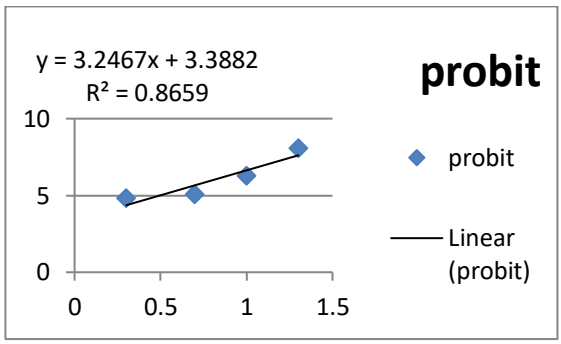


Figure 47: regression line: probits as a function the log doses at the Root (Roo EtOH) for 8h

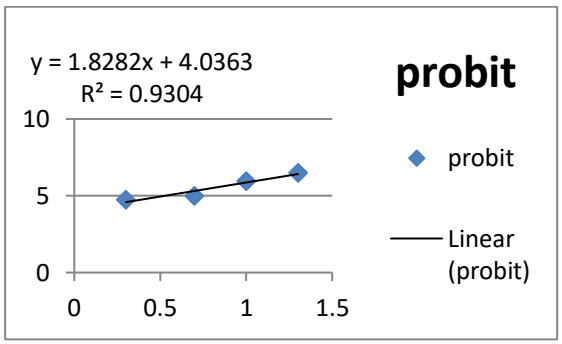


Figure 48: regression line: probits as a function the log doses at the Leaves (Lea EtOH) for 8h

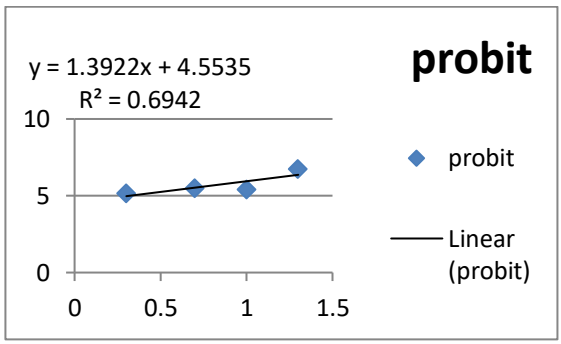


Figure 49: regression line: probits as a function the log doses at the Stem (Ste EtOH) for 8h

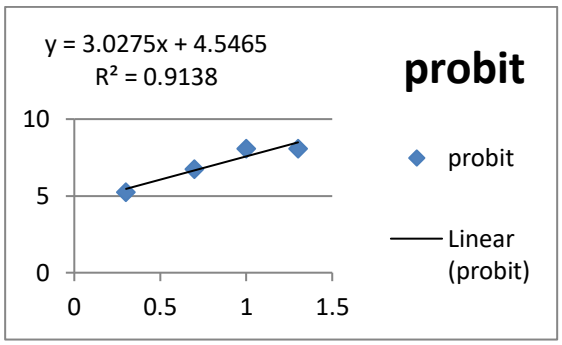


Figure 50: regression line: probits as a function of the log doses at the Root (Ro EtOH) for 12h

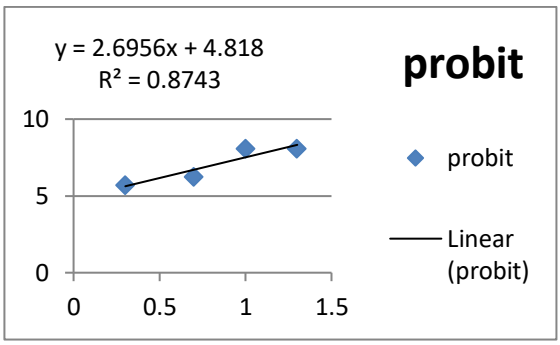


Figure 51: regression line: probits as a function of the log doses at the Leaves (Lea EtOH) for 12

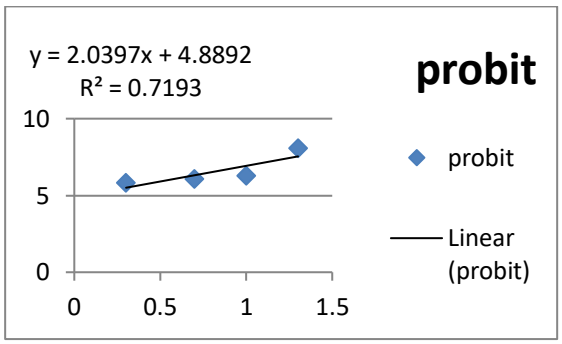


Figure 52: regression line: probits as a function Of the log doses at the Stem (Ste EtOH) for 12h

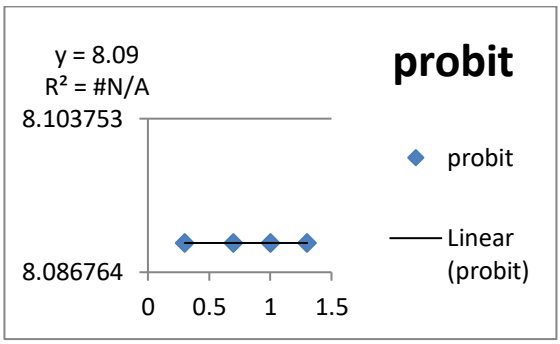


Figure 53: regression line: probits as a function Of the log doses at the Root (Ro EtOH) for 24h.

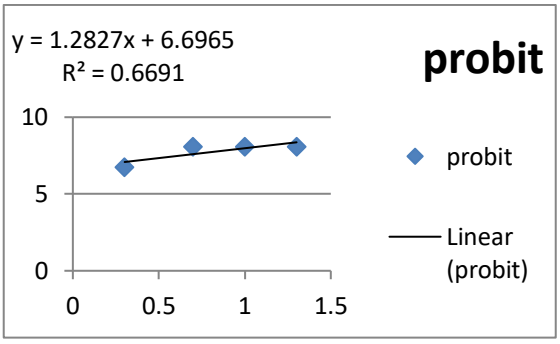


Figure 54: regression line: probits as a function Of the log doses at the Leaves (Lea EtOH) for 24h.

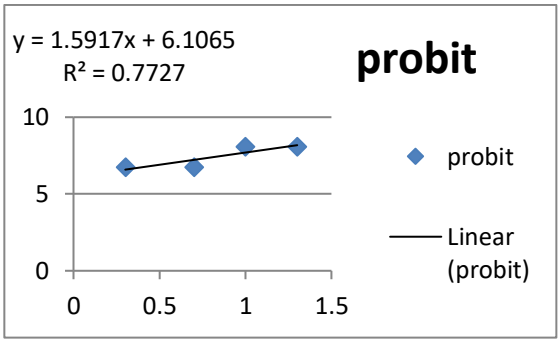


Figure 55: regression line: probits as a function Of the log doses at the Stem (Ste EtOH) for 24h.

Callosobruchus maculates

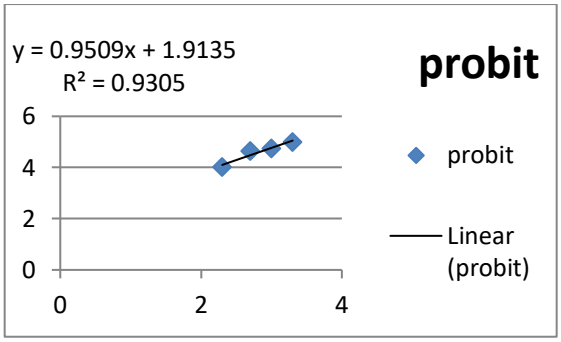


Figure 56: regression line: probits as a function Of the log doses at the Root (Ro Aq) for 8 h

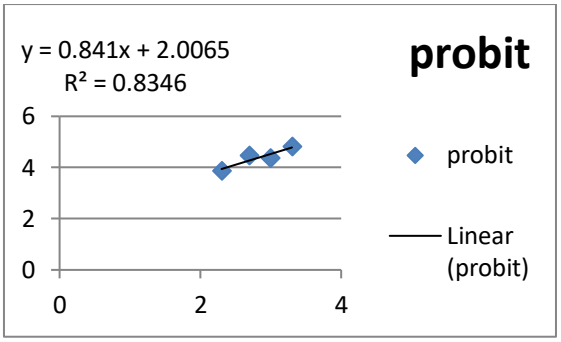


Figure 57: regression line: probits as a function the log doses at the leaves (Lea Aq) for 8 h.

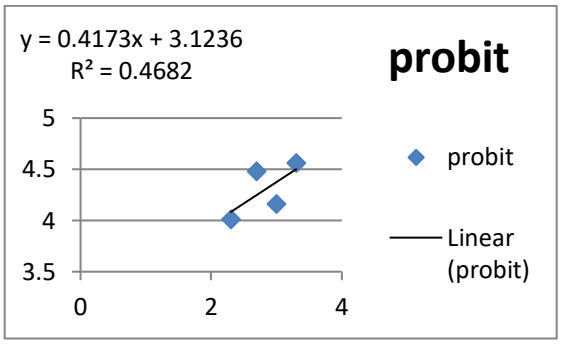


Figure 58: regression line: probits as a function Of the log doses at the Stem (Ste Aq) for 8 h.

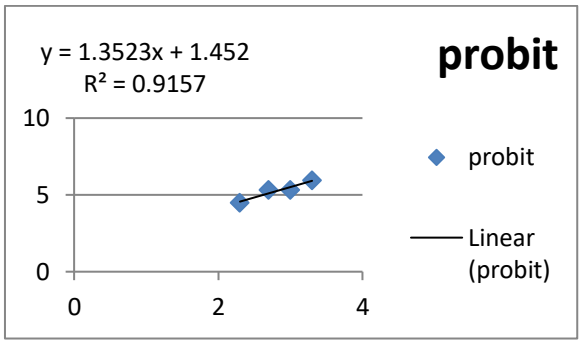
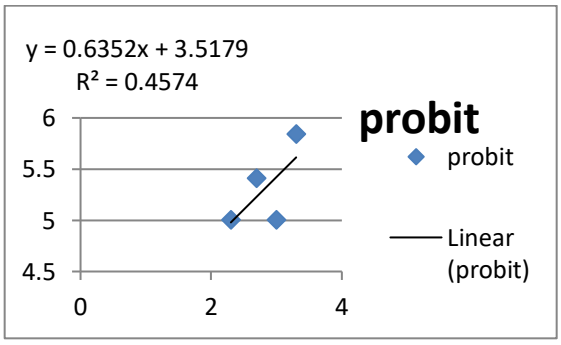


Figure 59: regression line: probits as a function Of the log doses at the Stem (Ste Aq) for 8 h.



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Figure 60: regression line: probits as function of the log doses at the Leaves (Lea Aq) for 12h

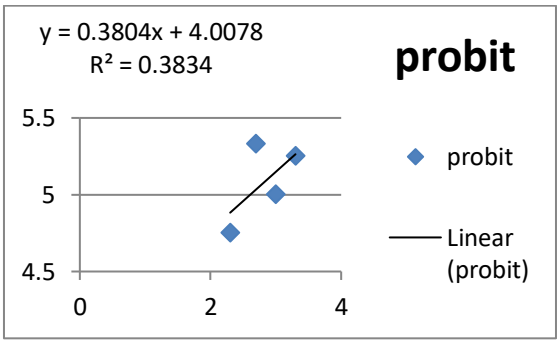


Figure 61: regression line: probits as function of the log doses at the Stem (Ste Aq) for 12h

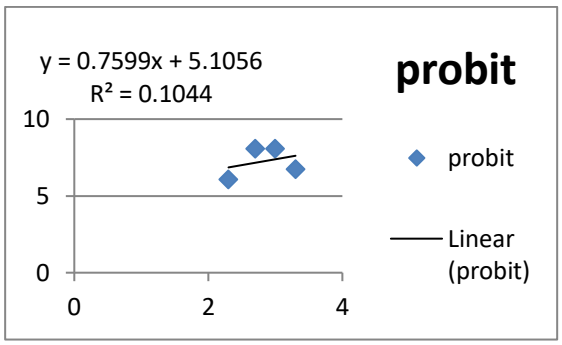


Figure 62: regression line: probits as a function Of the log doses at the Root (Ro Aq) for 24h

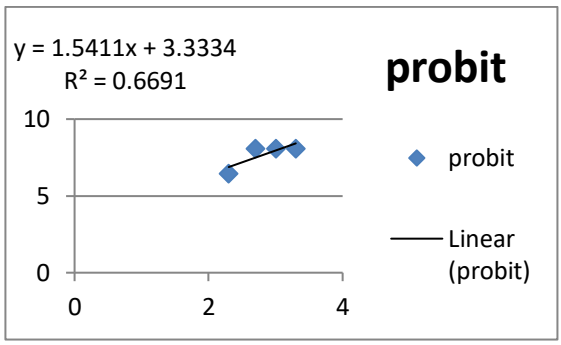


Figure 63: regression line: probits as a function of the log doses at the Leaves (Lea Aq) for 24h

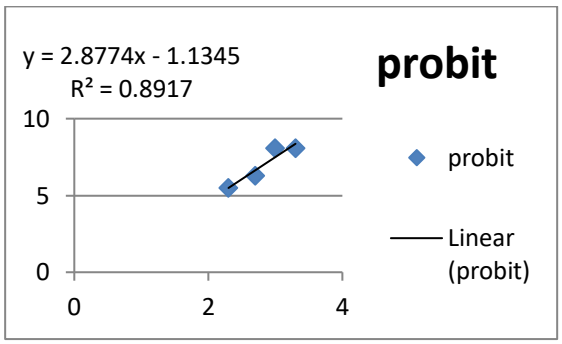


Figure 64: regression line: probits as a function Of the log doses at the Leaves (Lea Aq) for 24h

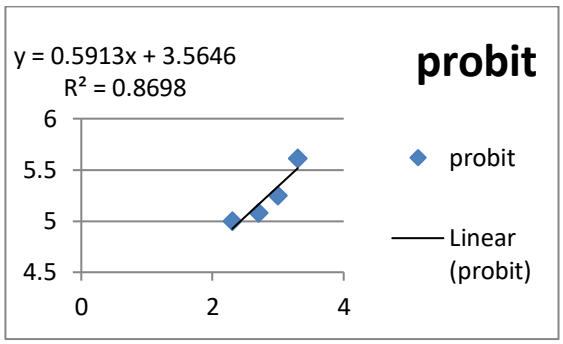


Figure 65: regression line: probits as a function of the log doses at the Root (Ro EtOH) for 8h

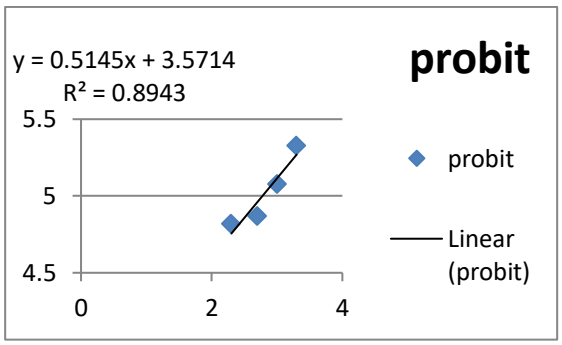


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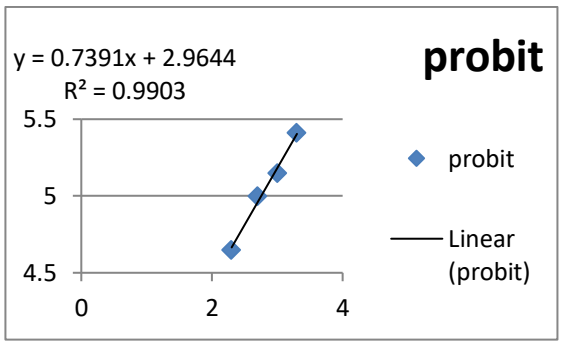


Figure 67: regression line: probits as a function Of the log doses at the Stem (Ste EtOH) for 8h

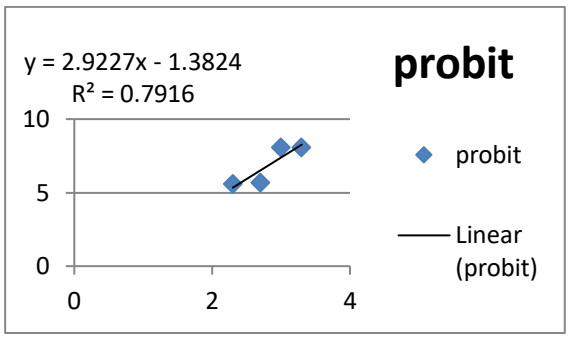


Figure 68: regression line: probits as a function of the log doses at the Root (Ro EtOH) for 12h

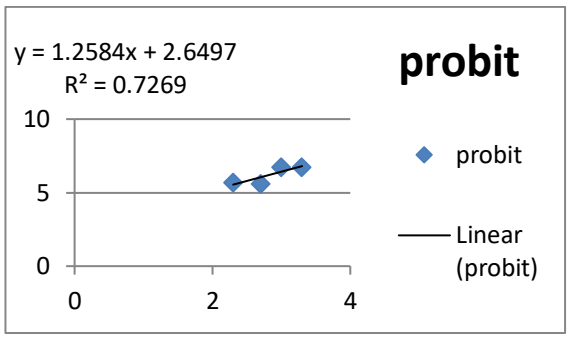


Figure 69: regression line: probits as a function of the log doses at the Leaves (Lea EtOH) for 12h

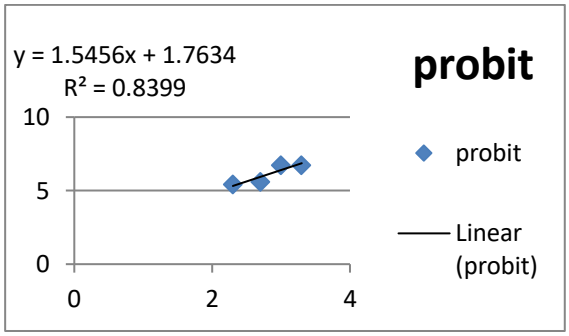


Figure 70: regression line: probits as a function of the log doses at the Stem (Ste EtOH) for 12h

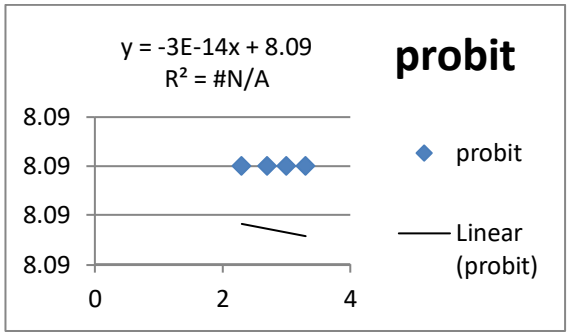


Figure 71: regression line: probits as a function Of the log doses at the Root (Ro EtOH) for 24h

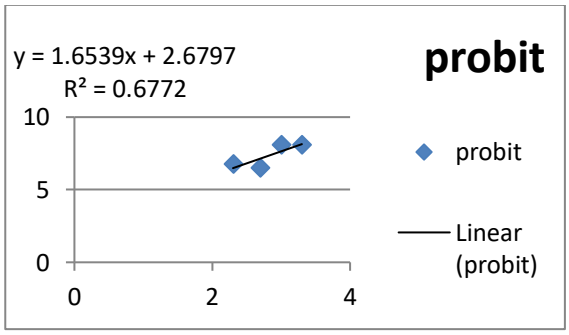


Figure 72: regression line: probits as a function of the log doses at the Leaves (Lea EtOH) for 24h.

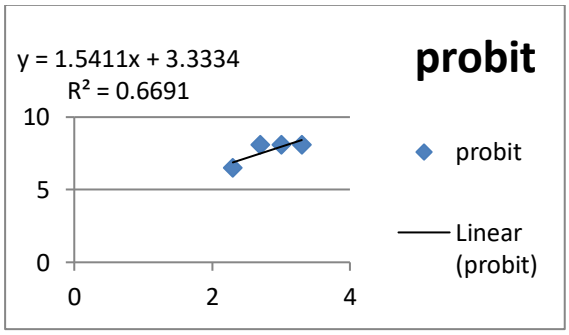


Figure 73: regression line: probits as a function of the log doses at the Stem (Ste EtOH) for 24h

Annexe 03: Estimation of the letal time (LT_{50})

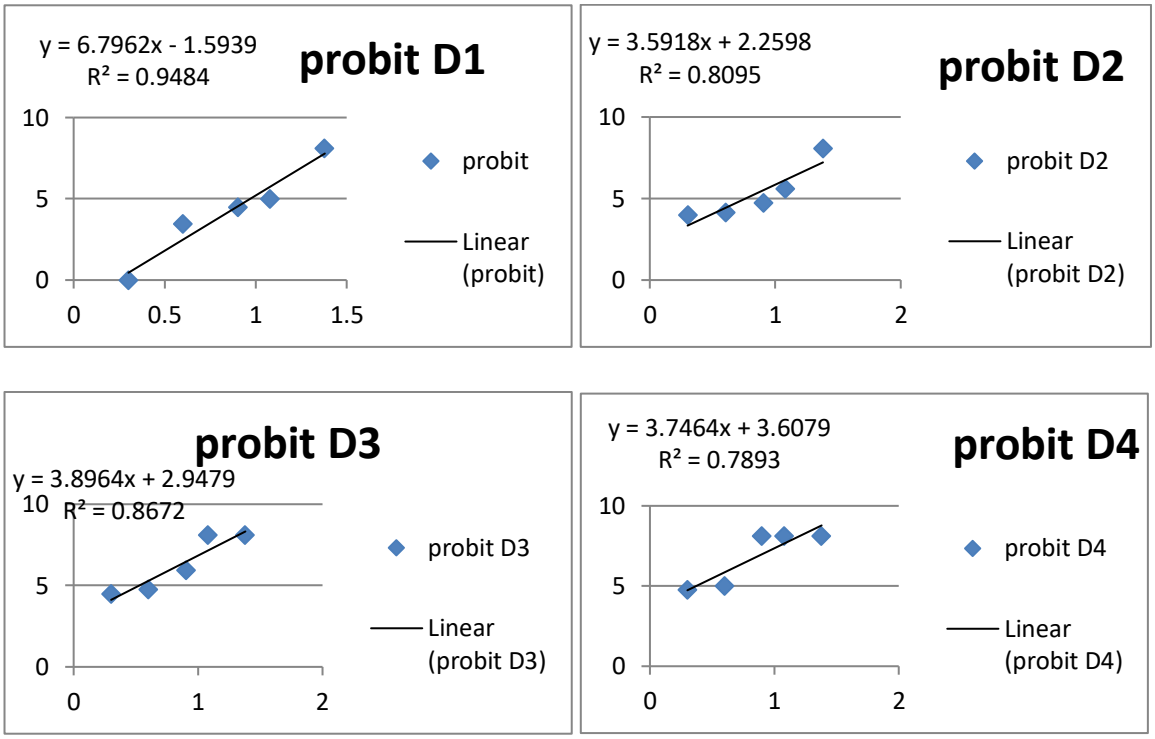


Figure 74 : Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Root (H2O).

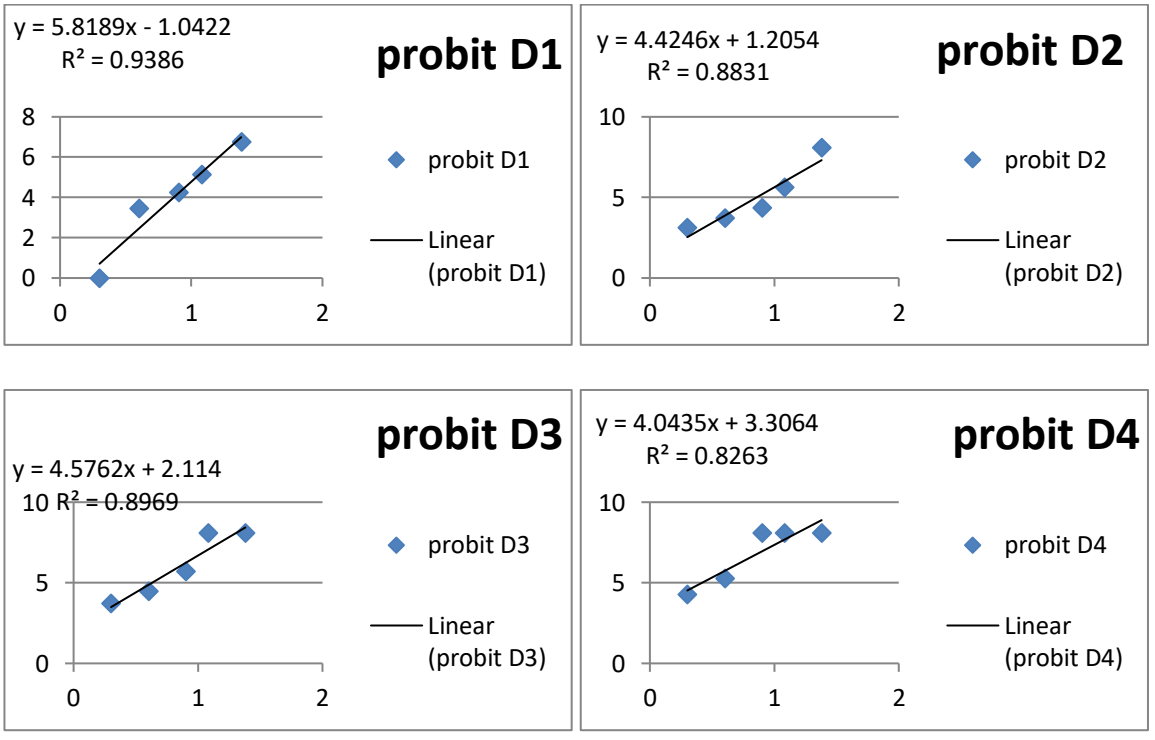


Figure 75: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Leaves (H2O).

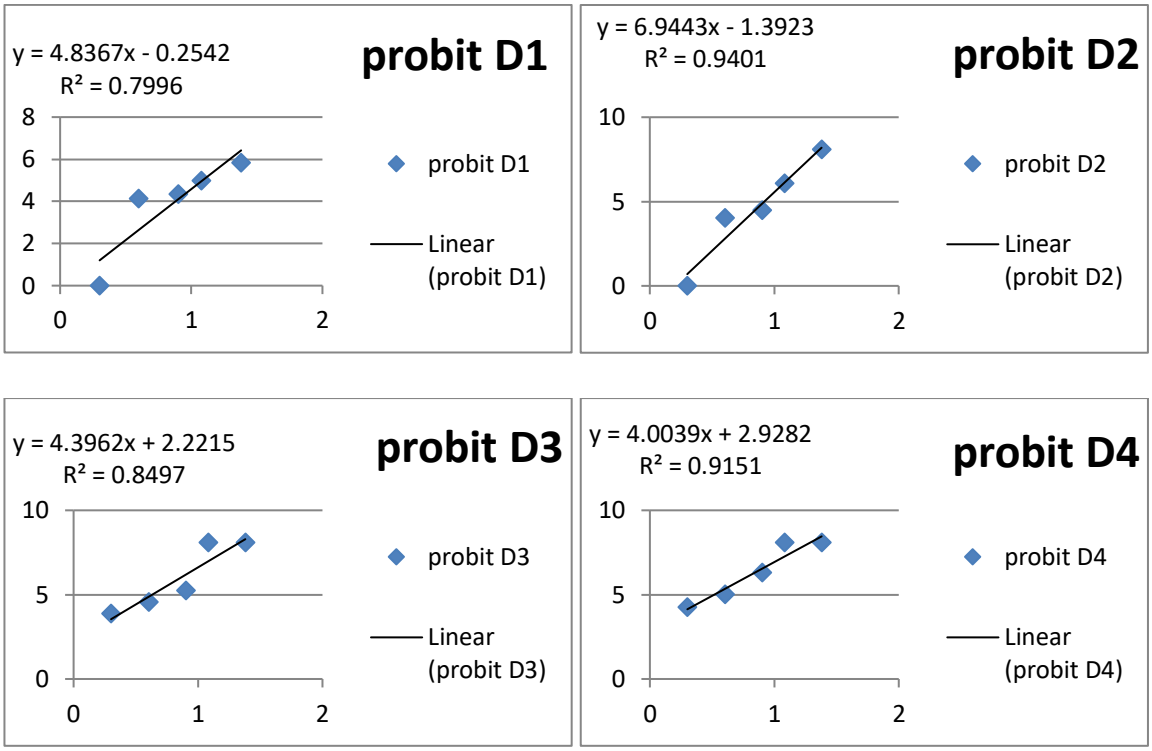


Figure 76 : Regression line: probits as a function of log time for D1, D2 , D3 and D4 in Stem (H2O).

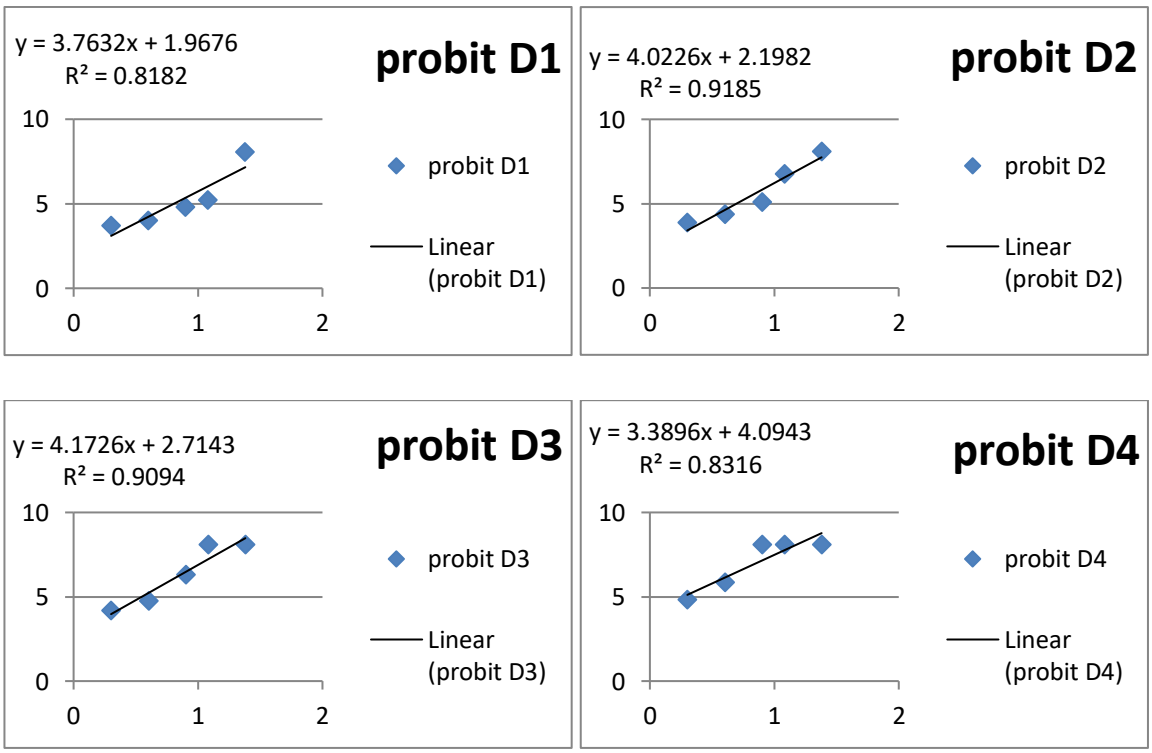


Figure 77: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Root (EtOH).

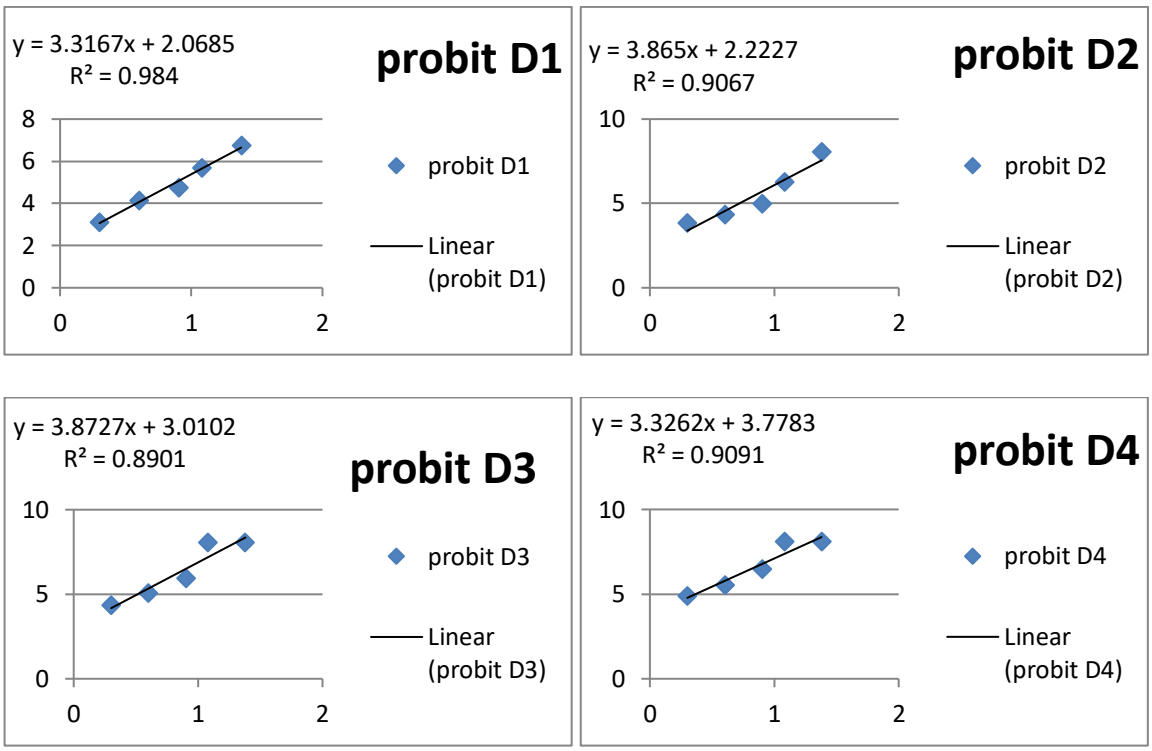


Figure 78: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Leaves (EtOH).

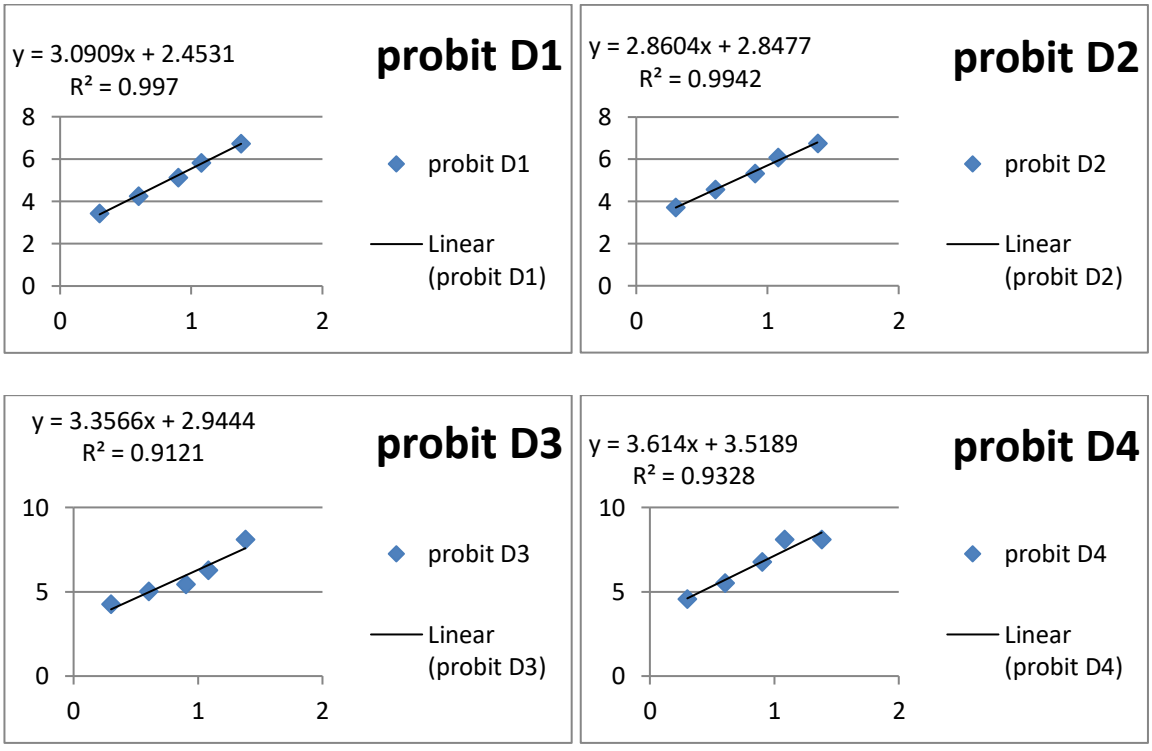


Figure 79: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Stem (EtOH).

*C.maculatus*are

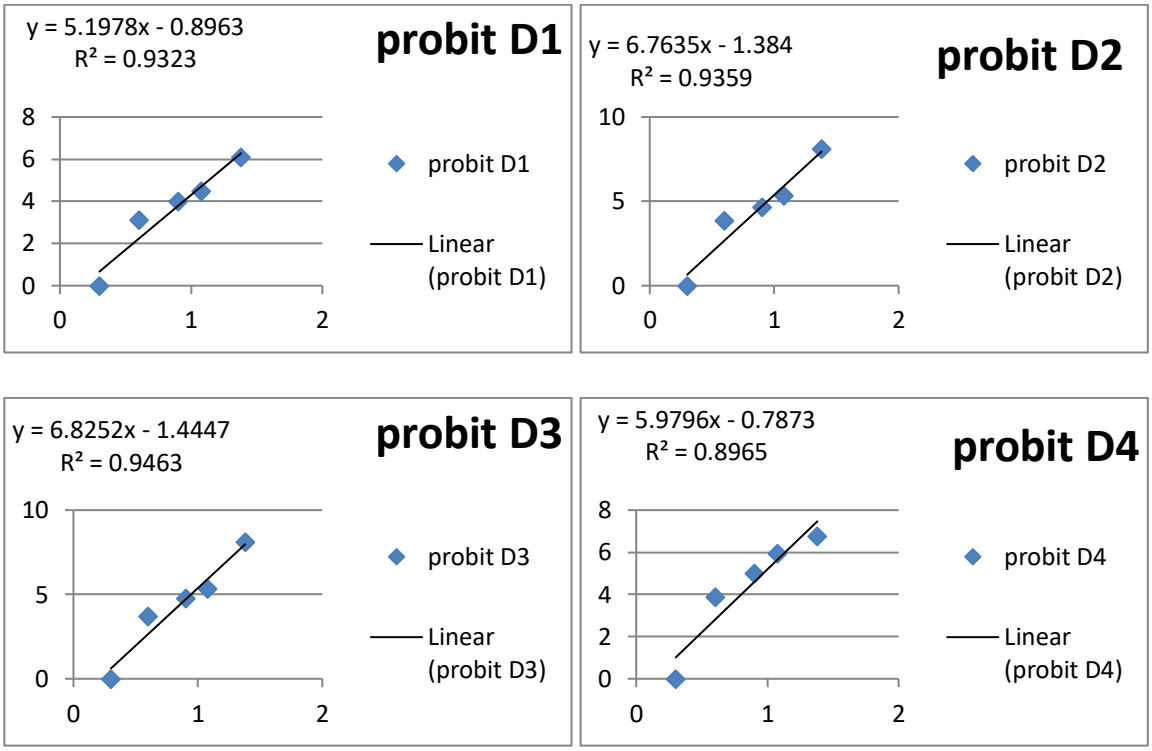


Figure 80: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Root (H_2O).

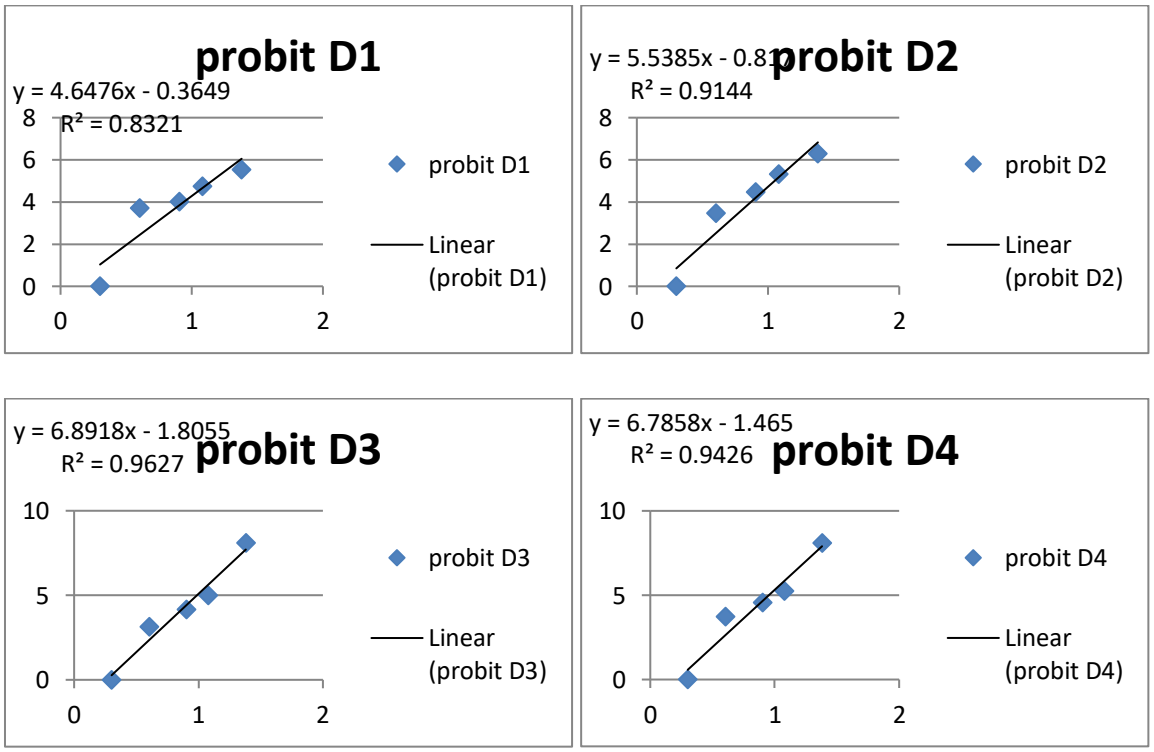


Figure 81: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Leaves (H2O).

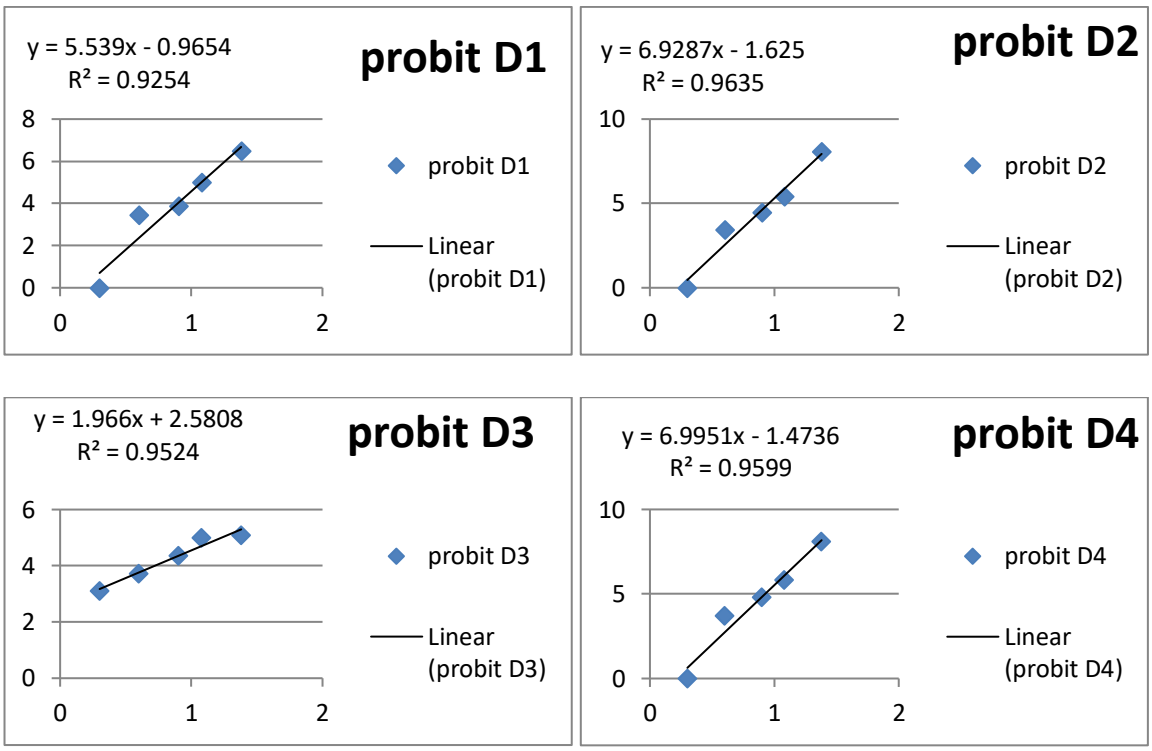


Figure 82: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Stem (H_2O).

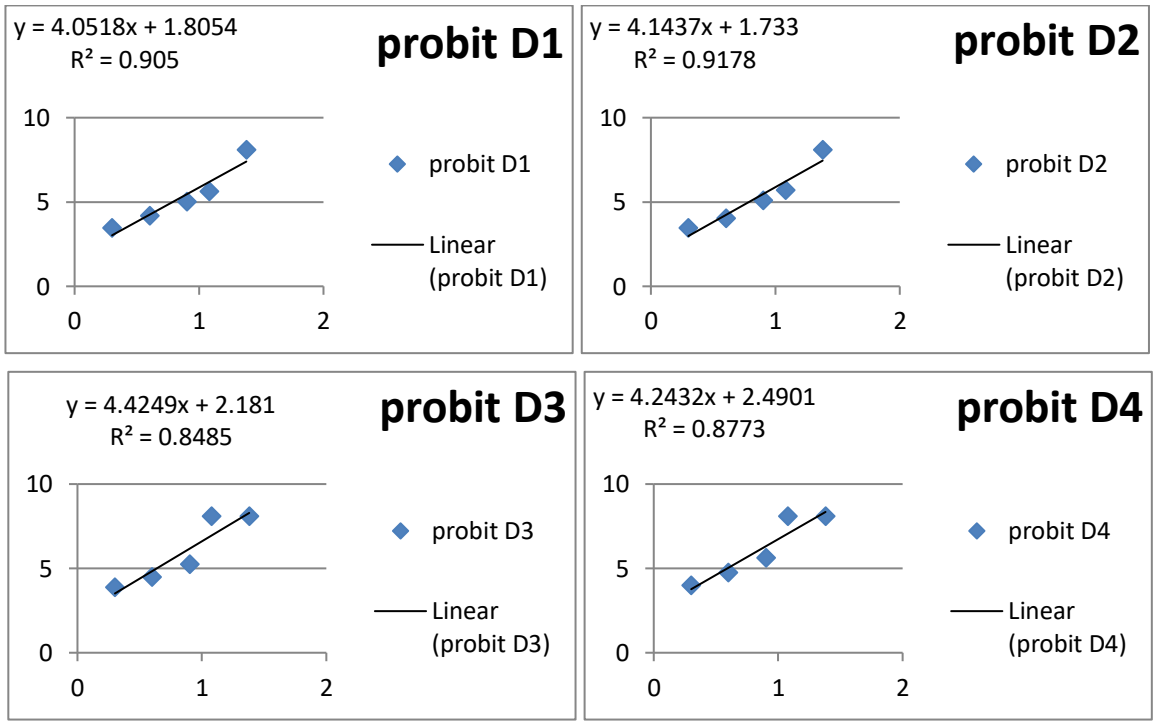


Figure 83: Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Root (EtOH).

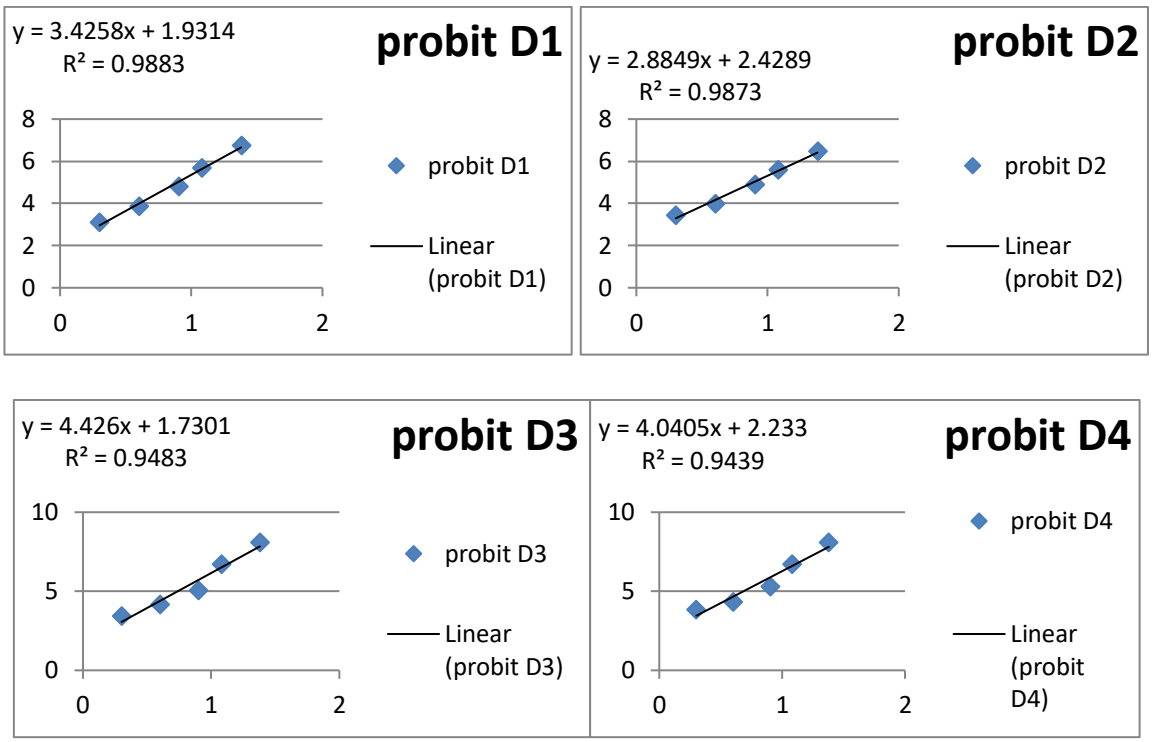


Figure 84 : Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Leaves (EtOH).

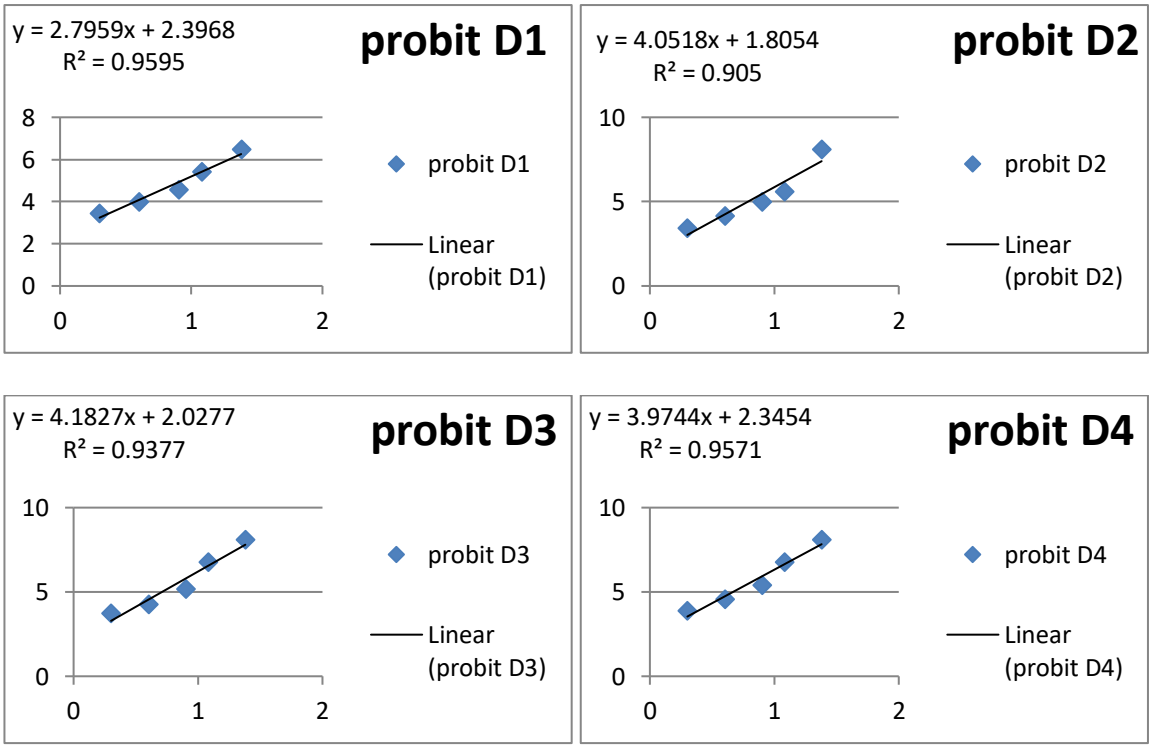


Figure 85 : Regression line: probits as a function of log time for D1 , D2 , D3 and D4 in Stem (EtOH).

Annexe 04: table of probit

%	0	1	2	3	4	5	6	7	8	9
0	____	2,67	2,95	3,12	3,25	3,36	3,45	3,52	3,59	3,66
10	3,72	3,77	3,82	3,87	3,92	3,96	4,01	4,05	4,08	4,12
20	4,16	4,19	4,23	4,26	4,29	4,33	4,36	4,39	4,42	4,45
30	4,48	4,5	4,53	4,56	4,59	4,61	4,64	4,67	4,69	4,72
40	4,75	4,77	4,8	4,82	4,85	4,87	4,9	4,92	4,95	4,97
50	5	5,03	5,05	5,08	5,1	5,13	5,15	5,18	5,2	5,23
60	5,25	5,28	5,31	5,33	5,36	5,39	5,41	5,44	5,47	5,5
70	5,52	5,55	5,58	5,61	5,64	5,67	5,71	5,74	5,77	5,81
80	5,84	5,88	5,92	5,95	5,99	6,04	6,08	6,13	6,18	6,23
90	6,28	6,34	6,41	6,48	6,55	6,64	6,75	6,88	7,05	7,33
	0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9
99	7,33	7,37	7,41	7,46	7,51	7,58	7,75	7,75	7,88	8,09