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THEME

Phytochemical Screening, Antioxidant, Anti-Inflammatory, and Insecticidal Activities of *Capsicum annuum* L. Extracts

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DEDICATION

"And say, 'Work, for Allah will see your deeds, and so will His Messenger and the believers.'"

Allah Almighty has spoken the truth.

First and foremost, all praise and gratitude are due to Allah, who blessed me with the strength, patience, and determination to complete this work and illuminated my path with knowledge and wisdom.

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Finally, to **everyone** who taught me even a single letter, my sincere gratitude and appreciation.

.

Hadil El Arabi Medkhel

DEDICATION

In the name of Allah, the Most Gracious, the Most Merciful.

“And say, ‘Work, for Allah will see your deeds, and [so will] His Messenger and the believers.’”

(At-Tawbah 9:105)

2026

With gratitude and pride, I dedicate this work to my beloved parents, **Chalbi Ammara** and **Naghmmouche Ali Khadidja**, whose love, sacrifices, support, and prayers have been the foundation of my success.

To my dear **brother** and beloved **sisters**, thank you for your constant encouragement, love, and support throughout this journey.

I also dedicate this work to **everyone** who believed in me and stood by my side during this important chapter of my life.

Chalbi Zainab

DEDICATION

“And say: Work, for Allah will see your deeds, and so will His Messenger and the believers.”

(Surah At-Tawbah, 9:105)

First and foremost, all praise and gratitude are due to Allah, whose blessings, guidance, and mercy have enabled me to complete this work.

To our beloved **Prophet Muhammad** ﷺ, the beacon of guidance and mercy.

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.

GAID SAFA

Abstract

The present study focuses on the biological and phytochemical investigation of Capsicum annuum L., aiming to perform a phytochemical screening of the plant extracts, evaluate their biological activities including antioxidant and anti-inflammatory properties, and assess their insecticidal activity against aphids. This work also includes a general overview of the chili pepper plant, its taxonomy, morphology, chemical composition, nutritional importance, and pharmaceutical applications. Special attention was given to bioactive compounds such as polyphenols, flavonoids, tannins, capsaicinoids, and carotenoids due to their significant biological activities. Experimentally, extracts were prepared and analyzed for their phenolic composition using standard phytochemical methods. The antioxidant activity was evaluated using DPPH and FRAP assays, while the anti-inflammatory potential was determined through the protein denaturation inhibition assay (BSA test). In addition, the insecticidal efficacy of the extracts was tested against aphids in order to assess their potential use as a natural biopesticide. The obtained results revealed that the extracts of Capsicum annuum L. contained considerable amounts of phenolic compounds, with total phenolic contents ranging from 2.20 ± 2.23 GAE/g extract. The extracts also exhibited significant antioxidant activity, as confirmed by the DPPH and FRAP assays, with inhibition percentages reaching 81.1% and reducing power values of 3.58 ± 0.04. Furthermore, promising anti-inflammatory effects were observed through the inhibition of protein denaturation, with inhibition rates varying according to extract concentration. In addition, the extracts demonstrated notable insecticidal activity against aphids, where the mortality rate increased progressively with both concentration and exposure time, reaching a maximum value of 96.66% after 2 hours. In conclusion, Capsicum annuum L. represents an important natural source of biologically active compounds with potential applications in pharmaceutical, agricultural, and food industries. These findings support the valorization of chili pepper extracts as eco-friendly alternatives for biological control and therapeutic purposes.

Keywords: *Capsicum annuum* L., polyphenols, antioxidant activity, anti-inflammatory activity, aphids, insecticidal activity, capsaicinoids.

الملخص

تتناول هذه الدراسة البحث البيولوجي والفيتوكيميائي لنبات Capsicum annuum L.، حيث تهدف إلى إجراء تحري فيتوكيميائي لمستخلصات النبات، وتقييم نشاطها البيولوجي المتمثل في النشاط المضاد للأكسدة والنشاط المضاد للالتهاب، بالإضافة إلى دراسة فعاليتها الحشرية ضد حشرات المنّ (الأفيد). كما يتضمن هذا العمل عرضًا عامًا لنبات الفلفل الحار من حيث تصنيفه العلمي، مورفولوجيته، تركيبه الكيميائي، أهميته الغذائية، وتطبيقاته الصيدلانية. وقد تم إيلاء اهتمام خاص للمركبات النشطة حيويًا مثل البوليفينولات، الفلافونويدات، التانينات، الكابسيسينويدات، والكاروتينويدات نظرًا لدورها الحيوي المهم. من الناحية التجريبية، تم تحضير المستخلصات وتحليل محتواها الفينولي باستخدام طرق فيتوكيميائية قياسية. وتم تقييم النشاط المضاد للأكسدة باستخدام اختباري DPPH وFRAP، في حين تم تحديد النشاط المضاد للالتهاب من خلال اختبار تثبيط تمسخ البروتين (اختبار BSA). بالإضافة إلى ذلك، تم اختبار الفعالية الحشرية للمستخلصات ضد حشرات المنّ بهدف تقييم إمكانية استخدامها كمبيد حيوي طبيعي. أظهرت النتائج المتحصل عليها أن مستخلصات Capsicum annuum L. تحتوي على كميات معتبرة من المركبات الفينولية، حيث تراوحت نسبة البوليفينولات الكلية حوالي 2.20 ± 2.23 ملغ مكافئ حمض الغاليك/غ من المستخلص. كما أبدت المستخلصات نشاطًا مضادًا للأكسدة مهمًا، تم تأكيده من خلال اختباري DPPH وFRAP، بنسبة تثبيط بلغت 81.1% وقدرة اختزالية قدرت بـ 3.58 ± 0.04. علاوة على ذلك، لوحظت تأثيرات واعدة مضادة للالتهاب من خلال تثبيط تمسخ البروتين، مع اختلاف نسب التثبيط حسب تركيز المستخلص. كما أظهرت المستخلصات فعالية حشرية ملحوظة ضد حشرات المنّ، حيث ارتفعت نسبة النفوق تدريجيًا مع زيادة التركيز ومدة التعرض، لتصل إلى 96.66% بعد ساعتين. في الختام، يُعد Capsicum annuum L. مصدرًا طبيعيًا مهمًا للمركبات النشطة حيويًا ذات تطبيقات محتملة في المجالات الصيدلانية والزراعية والغذائية. وتدعم هذه النتائج إمكانية تثمين مستخلصات الفلفل الحار كبدائل بيئية فعالة في المكافحة الحيوية والاستخدامات العلاجية.

الكلمات المفتاحية: *Capsicum annuum* L.، البوليفينولات، النشاط المضاد للأكسدة، النشاط المضاد للالتهابات، حشرة المنّ، النشاط المبيد للحشرات، الكابسيسينويدات.

Résumé

La présente étude porte sur l'investigation biologique et phytochimique de *Capsicum annuum* L., ayant pour objectifs de réaliser un criblage phytochimique des extraits de la plante, d'évaluer leurs activités biologiques notamment antioxydante et anti-inflammatoire, ainsi que d'étudier leur activité insecticide contre les pucerons. Ce travail comprend également une présentation générale du piment, incluant sa taxonomie, sa morphologie, sa composition chimique, son importance nutritionnelle ainsi que ses applications pharmaceutiques. Une attention particulière a été accordée aux composés bioactifs tels que les polyphénols, les flavonoïdes, les tanins, les capsaïcinoïdes et les caroténoïdes, en raison de leurs importantes activités biologiques. Sur le plan expérimental, les extraits ont été préparés et analysés afin de déterminer leur composition phénolique à l'aide de méthodes phytochimiques standard. L'activité antioxydante a été évaluée par les tests DPPH et FRAP, tandis que le potentiel anti-inflammatoire a été déterminé par le test d'inhibition de la dénaturation des protéines (test BSA). Par ailleurs, l'efficacité insecticide des extraits a été testée contre les pucerons afin d'évaluer leur potentiel en tant que biopesticides naturels. Les résultats obtenus ont montré que les extraits de *Capsicum annuum* L. contiennent des quantités importantes de composés phénoliques, avec une teneur totale en polyphénols variant autour de 2,20 ± 2,23 mg EAG/g d'extrait. Les extraits ont également présenté une activité antioxydante significative, confirmée par les tests DPPH et FRAP, avec des pourcentages d'inhibition atteignant 81,1 % et une capacité réductrice de 3,58 ± 0,04. En outre, des effets anti-inflammatoires prometteurs ont été observés via l'inhibition de la dénaturation des protéines, avec des taux variables selon la concentration des extraits. Par ailleurs, une activité insecticide notable contre les pucerons a été mise en évidence, avec une mortalité augmentant progressivement en fonction de la concentration et du temps d'exposition, atteignant un maximum de 96,66 % après 2 heures. En conclusion, *Capsicum annuum* L. constitue une source naturelle importante de composés bioactifs présentant des applications potentielles dans les domaines pharmaceutique, agricole et agroalimentaire. Ces résultats soutiennent la valorisation des extraits de piment comme alternatives écologiques pour la lutte biologique et les usages thérapeutiques.

Mots-clés : *Capsicum annuum* L., polyphénols, activité antioxydante, activité anti-inflammatoire, pucerons, activité insecticide, capsaïcinoïdes.

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INTRODUCTION

Introduction

The use of plant extracts in the control of agricultural pests has become one of the modern approaches that has attracted considerable attention in recent years due to its biological effectiveness and environmental safety compared with conventional chemical pesticides. Pepper pepper is considered one of the plants rich in biologically active secondary metabolites, such as capsaicin, phenolics, flavonoids, and tannins, which have demonstrated a significant effect against many harmful insects, particularly aphids aphids. These compounds inhibit insect feeding behavior and interfere with their growth and reproduction, making pepper extracts a promising option within biological control programs and natural alternatives to chemical pesticides (El-Beltagi *et al*., 2021).

Aphids are regarded as one of the most destructive agricultural pests affecting numerous crops, including pepper, as they feed on plant sap, causing weak plant growth, leaf deformation, and reduced crop productivity. In addition, aphids play a major role in transmitting several dangerous plant viruses (Gebretsadik *et al*., 2023). The excessive use of chemical pesticides has led to the emergence of resistant aphid populations, as well as negative impacts on the environment, beneficial organisms, and human health, which has encouraged researchers to develop safer and more sustainable natural pest control methods (Deguine *et al*., 2021).

In this context, several studies have demonstrated that pungent plant extracts, especially those derived from pepper fruits rich in capsaicin, possess effective insecticidal activity against aphids. These extracts affect the insect nervous system and reduce feeding activity and reproduction. Moreover, they are characterized by ease of preparation, low cost, and biodegradability without leaving toxic residues in the environment (Pavela & Benelli, 2016). Therefore, the use of pepper extracts as a form of botanical biological control has become one of the promising solutions that can be integrated into integrated pest management programs.

Accordingly, this research aims to investigate the effectiveness of pepper extract in controlling aphids and to evaluate its impact on reducing infestation and improving plant growth, thereby contributing to the development of environmentally friendly natural alternatives that reduce dependence on conventional chemical pesticides.

This study is divided into two main parts:

The first part consists of a theoretical section that provides a general overview of pepper plants (*Capsicum annuum* L.), including their botanical characteristics, chemical composition,

and biologically active compounds, particularly capsaicin and phenolic substances. It also discusses aphids, their biological characteristics, harmful effects on crops, and the role of botanical extracts in biological pest control.

The second part is devoted to the experimental section, which focuses on the preparation of pepper extract and the evaluation of its biological activity against aphids. This part includes the materials and methods used in the experiments, followed by the presentation and discussion of the obtained results regarding the insecticidal effect of the extract and its potential use as a natural alternative to chemical pesticides

FIRST PART

Bibliographical Synthesis

CHAPTER I

General Overview of Chili Pepper Plant (Capsicum annuum L.)

I.1. Taxonomy and Terminology

Capsicum annuum L. is a dicotyledonous flowering plant commonly grown worldwide, with many general names in English, such as hot pepper, chili, chilli or chile pepper, and as well sweet pepper and bell pepper. Sometimes the plant is just called pepper, which however is often reserved for the earlier known Asian Piper nigrum (black pepper, white pepper) in the family Piperaceae. The pre-Columbian, indigenous Nahua (Aztec) Amerindian name for the plant was transcribed as chilli or chili, and the usual name in Spanish is chile, which results in the plurals of chillies, chilies, and chiles (Bosland, 1996). Other broad names for *C. annuum* relate more to particular varieties or strains, culinary uses, and ripeness, such as jalapeño, Cayenne, pimento (pimiento), paprika, red, and green peppers. Furthermore, four other less commonly cultivated Capsicum species are also considered chile peppers, and two of these species are similar and closely related to *C. annuum*.

Capsicum annuum is usually grown as a herbaceous annual in temperate areas. However, ecologically it is a perennial shrub in tropical areas (which may live a few years to a few decades), and it can be grown as a perennial in climate-controlled greenhouses. This species includes the vast majority of the cultivated pungent and non-pungent (sweet) Capsicum peppers in temperate as well as some tropical areas. In the species *C. annuum* throughout the world, there is phenotypic diversity in plant habit and especially in shapes, sizes, colours, pungency, and other qualities of the fruits (Andrews, 1995, 1998, 1999; DeWitt and Bosland, 1996, Greenleaf, 1986). This immense horticultural, agricultural and biological diversity has helped to make *C. annuum* globally important as a fresh and cooked vegetable (e.g. for salads, warm dishes, pickled) and a source of food ingredients for sauces and powders and as a colourant, which is used as well in cosmetics (Andrews, 1995, 1999; Bosland, 1994; Bosland and Votava, 2000). Moreover, the species is used medicinally and medically, and provides the ingredient for a non-lethal deterrent or repellent to some human and animal behaviours (Krishna De, 2003; Cordell and Araujo, 1993; Palevitch and Craker, 1995; Cronin, 2002; Cichewicz and Thorpe, 1996; Reilly *et al*., 2001). Chili peppers are also cultivated ornamentally especially for their brightly glossy fruits with a wide range of colours.

Chili pepper comprises numerous chemicals including steam-volatile oil, fatty oils , capsaicinoids, carotenoids, vitamins, protein, fibre, and mineral elements (Bosland and Votava, 2000; Krishna De, 2003). Many chili pepper constituents have importance for nutritional value, flavour, aroma, texture, and colour. The ripe fruits are especially rich in vitamin C (Osuna-García *et al*., 1998; Marin *et al*., 2004). The two chemical groups of greatest interest are the capsaicinoids and the carotenoids. The capsaicinoids are alkaloids that give hot chili peppers

their characteristic pungency. The rich supply of carotenoids contributes to chili peppers nutritional value and colour As shown in the (Figure I.1) (Britton and Hornero-Méndez, 1997; Hornero-Méndez *et al*., 2002; Pérez-Gálvez *et al*., 2003)

Figure I.1: *Capsicum annuum* L.

I.2. Family Solanaceae:

The genus Capsicum L. is in the ripe family Solanaceae, which includes as food the potato (Solanum tuberosum), tomato (Lycopersicon esculentum Solanum lycopersicum), tree tomato (Cyphomandra betacea or Solanum betaceum), eggplant (Solanum melongena), African eggplants (Solanum macrocarpon, S. aethiopicum), husk or strawberry tomato (Physalis pruinosa) and Cape gooseberry (P. peruviana), as well as tobacco (Nicotiana tabacum), medicinal plants such as deadly nightshade (Atropa belladonna) and Datura stramonium, ornamentals such as tree daturas (Brugmansia) (which are also hallucinogenic) and Petunia, and weeds such as black nightshade (Solanum nigrum) (Knapp, 2002; Hunziker, 2001; George, 1985), Capsicum is in the subfamily Solanoideae and tribe Capsiceae (Olmstead *et al*., 1999; Knapp, 2002; Knapp *et al*., 2004; Hunziker, 2001). The genus Capsicum consists of about 25 wild and 5 domesticated species (Table 2) (IBPGR, 1983; Eshbaugh, 1993; Bosland and Votava, 2000).

I.3. Scientific classification:

According to Bentham and Hooker's Classification system As shown in the table:

Table I.1. Classification of *Capsicum annuum*

Kingdom	Plantae
Sub kingdom	Phanerogams
Division	Angiosperms
Class	Dicotyledonae
Sub class	Gamopetalae
Series	Bicarpellatae
Order	Polemoniales
Family	Solanaceae
Genus	Capsicum
Species	*C. annuum*

I.4. Types of Capsicum and their geographical distribution

The species of Capsicum were listed with their seemingly natural distributions by the International Board for Plant Genetic Resources (IBPGR, 1983) and updated by Eshbaugh (1993), Hernández-Verdugo *et al*. (1999) and Bosland and Votava (2000); they are listed in Table 2 as currently understood. There is modest uncertainty on the generic limits of Capsicum and more uncertainty on its tribal relatives (which at minimum include Lycianthes) (Eshbaugh, 1993; Hunziker, 2001; Knapp, 2002), and a lack of consensus on the number and in a few cases the botanical names of the known Capsicum species, and on the truly natural distributions of several species (rather than confounding naturalised with native populations) As shown in the table :

Table I.2. The species of Capsicum and their known or apparently natural distributions; those with haploid chromosome number n 13 rather than 12 are noted (Tong and Bosland, 2003). The five domesticated species are grouped into the *C. annuum* complex (3 spp.) (Ca), the C. baccatum complex (Ca), and the C. pubescens complex (C)

Species	Known or probable natural distribution
C. annuum L. (CA)	southern USA to Colombia
C. baccatum L. (C)	Peru, Bolivia, Paraguay, Argentina and Brazil
C. buforum Hunz Species	southern Brazil
C. campylopodium Sendtner; n=13	southern Brazil
C. cardenasii Heiser & P.G. Smith (Cp)	northeastern Bolivia

C. chacoense Hunz. (Ca or CA)	Argentina, Paraguay and Bolivia
C. chinense Jacq. (CA)	(northern) Amazonian South America
C. coccineum (Rusby) Hunz.	Bolivia and Peru
C. cornutum (Hiern) Hunz.	southern Brazil
C. dimorphum (Miers) Kuntze	Colombia
C. dusenii Bitter	southeastern Brazil
C. eximiun Hunz. (C)	Bolivia and northern Argentina
C. flexuosum Sendtner	Argentina, Brazil and Paraguay
C. frutescens L. (C)	western Amazon (Colombia to Peru)
C. galapagoense Hunz. (CA)	Galápagos Islands (Ecuador)
C. geminifolium (Dammer) Hunz.	Colombia and Ecuador.
C. hookerianum (Miers) Kuntze	Ecuador and northwestern Peru
C. lanceolatum (Greenman) Morton & Standley; 13	Honduras, Guatemala and Mexico
C. leptopodum (Dunal) Kuntze	Brazil
C. minutiflorum (Rusby) Hunz.	Argentina, Paraguay and Bolivia
C. mirabile Mart. ex Sendtner; n = 13	southern Brazil
C. parvifolium Sendtner	northeastern Brazil, Venezuela and Colombia
C. praetermissum Heiser & P.G. Smith (Ca)	southern Brazil
]synonym *C. baccatum* var. praetermissum. (Heiser & P.G. Smith) Hunz[.	Bolivia to Colombia [only in cultivation] Mexico to Peru
C. pubescens Ruiz & Pavón (CP)	southern Brazil, Paraguay and Argentina.
C. rhomboideum (Dunal) Kuntze	northwestern Peru
[synonym *C. ciliatum* (Kunth) Kuntze]; n = 13	south-central Peru
C. schottianum Sendtner; n = 13	southern Brazil
C. scolnikianum Hunz.	
C. tovarii Eshbaugh, P.G. Smith & Nickrent (C)	
C. villosum Sendtner	

Source: Tong and Bosland, 1999; Walsh and Hoot, 2001; Jarret and Dang, 2004; Ryzhova and Kochieva, 2004

I.5. Reproductive organs (morphology, development), fertilisation, dispersal and germination

I.5.1 Tree:

It is shrubby perennial herb. Their habits are annual sub woody plants which attain up to 65cm or more in height."

I.5.2 Leaves:

The leaves are simple, glabrous, lanceolate to ovate with apex being acately acuminate and the base being cuneate abruptly acute and petiolate.

I.5.3 Flower:

Capsicum annuum starts flowering at the axil of the first branching node, with subsequent flowers forming at each additional node (Bosland and Votava, 2000). Usually *C. annuum* has a solitary flower at the axil (Bosland and Gonzalez, 1994), although some accessions have a few clustered flowers between which there are short internodes (Smith and Heiser, 1951) (Table 3). Flower differentiation is not affected by daylength (Cochran, 1942). The most important factor determining differentiation is air temperature, especially at night (Bosland and Votava, 2000; Aloni *et al*., 1999; Rylski, 1972).

The Capsicum flower is bisexual, hypogynous and usually pentamerous (Bosland and Votava, 2000). The flowers are complete, with calyx, corolla, and male and female sex organs. The diameter of a *C. annuum* flower is 9-15 mm. The Capsicum calyx is broadly campanulate, ribbed, about 2 mm long, and truncate or undulate to weakly or prominently dentate with 5-7 teeth. The short-tubed corolla is rotate in most Capsicum species, with usually 5 but sometimes 6-7 (-8) petals in some species. The number of corolla lobes and stamens is equal. Typically the flowers have 5 stamens; the filaments are white or violet depending on the species (or variety), with the usually connivent to free anthers varying from bluish-purplish to yellow and white depending on the species (e.g. Table 3) (Dharamadhaj and Prakash, 1978). The pistil comprises an ovary of 2-3 (-4) carpels that is 2-5 mm long and 1.5-5 mm in diameter, a style 3.5-6.5 mm long, and a capitate papillate stigma slightly wider than the style. The style extends well beyond to just beyond the anthers or may be even with them, or it may be slightly exceeded by the anthers.

The daily start of anthesis apparently is controlled by daylength (Aleemullah *et al*., 2000). The corolla typically opens within the first 3 hours after sunrise, and the petals remain open for less than a day; there also can be a unripe peak of anthesis in the afternoon. Hirose (1957) found dehiscence of the anthers to occur late in the morning, between 10 am and noon. The

anthers open lengthwise from typically 1 hr after the flower opens to even 10 hrs afterward, but they frequently fail to dehisce entirely, or may dehisce the next morning if the flower opens in late afternoon (Aleemullah *et al*., 2000; cf. Horner and Wagner, 1992). Depending on the environmental conditions and variety, the period of receptivity of the stigma is 5-8 days, from several days before anthesis to fewer days afterwards, with maximum fertility on the day of anthesis As shown in the Figure **I.2** (Cochran and Dempsey, 1966; Barai and Roy, 1986; Aleemullah *et al*., 2000)

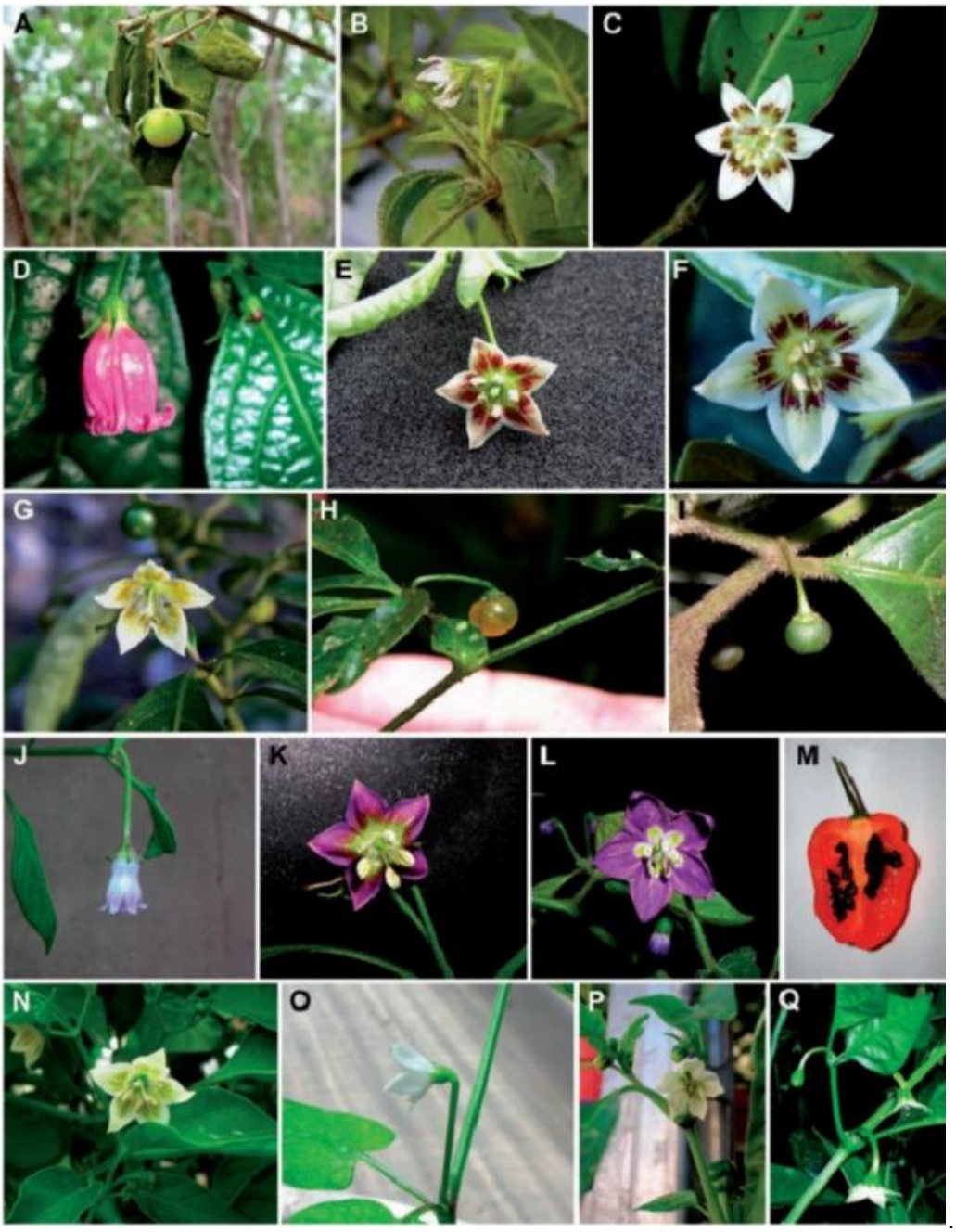

Figure I.2. Flowers and fruits of the Longidentatum (A), Atlantic (B–I), Purple Corolla (J–K), Pubescens (L, M), Baccatum (N, O) and Annuum (P, Q) clades. (A)

Capsicum longidentatum fruit. (B) Capsicum cornutum flower with stellate corolla and geniculate pedicel. (C) Capsicum pereirae flower with spotted stellate corolla.

(D) Capsicum friburgense urceolate-campanulate corolla and pedicel geniculate. (E) Capsicum mirabile stellate corolla with dark red spots. (F)

Capsicum hunzikerianum flower with spotted stellate corolla. (G, H) Capsicum sp. nov. (GEB & CCG 3637) stellate corolla with golden-green spots (G) and mature

greenish-golden yellow fruit, without well-developed calyx teeth (H). Note the different patterns of spots in the corolla in C and E–G. (I) Capsicum villosum var.

muticum immature fruit; note the absence of well-developed teeth. (J) Capsicum cardenasii pendant flower with shortly tubular corolla and non-geniculate flowering

pedicel. (K) Capsicum eximium flower with stellate corolla. (L, M) Capsicum pubescens flower (L) and longitudinal section of a mature fruit showing ripe blackish

brown seeds (M). (N) Capsicum baccatum var. pendulum flower showing the distinctive green spots in the corolla. (O) Capsicum chacoense flower showing immaculate white corolla and geniculate flowering pedicel. (P) *Capsicum annuum* var. annuum flowering and fruiting branch showing typical white corolla and entire calyx

without well-developed teeth. (Q) Capsicum chinense flowering branch showing pendant flowers with non-geniculate flowering pedicels and entire calyx without

well-developed teeth. Photos by G. Barboza (A, B, F), M. Sterpetti (C, D) and C. Carrizo Garcıa (E, G–Q).

I.5.4 Pollen and fertilisation:

The pollen grains of chili pepper are the intermediate maturity stage to light yellow, subspheroidal, pitted, and tricolporate with longitudinal grooves (Bosland and Votava, 2000). The plant has about 1-1.5 mg of pollen per flower (Quagliotti, 1979), with 11,000-18,000 pollen grains in a single anther (Hirose, 1957). Air temperature has a ripe effect on pollen formation and viability. Temperatures above 30°C occurring 15 days prior to anthesis cause pollen sterility (Cochran, 1938), and night temperatures of 10 ± 2 °C reduce the number and germinability of pollen grains (Shaked *et al*., 2004). The optimal temperature for pollen germination is 20-25°C. Pollen tube growth from the stigma to the egg has been reported to take 6 to 42 hrs. In detailed anatomical studies, Cochran (1938) found that fertilisation occurred 42 hrs after pollination in

plants grown at 27°/21°C, whereas Kato (1989) found that 36 hrs were needed for the fertilisation process

Male sterility is found in Capsicum, controlled by cytoplasmic and nuclear genes (Shifriss, 1997; Wang *et al*., 2004; Kalloo *et al*., 2002). In plants of both types, the anthers may be unripe and shrunken and blue-violet, with little or no viable pollen (Wien, 1997), or there may be no anthers (Derera *et al*., 2005).

I.5.5 Fruit:

There is extensive diversity in fruit shape, size, wall thickness and fleshiness, colour and pungency (Andrews, 1995; IPGRI *et al*., 1995), determined by genetic and environmental factors. Among the innumerable varieties of *C. annuum*, the diversification of shapes of the pod (fruit) is strikinge.g. blocky (or lantern- or bell-shaped), globose, oblong (sausage-shaped), ovoid, conical, cylindrical, banana-like (curved); and smooth, grooved, lumpy or wrinkled. The length of the pod varies from less than 1 to 32.5 cm. The pedicel length also varies in different pod types (over several cm), and the fruit may be erect to pendent (deflexed). Fruit colours range from green, yellow, orange and red to purple, brown, black, and white as well. Some of the genetics of fruit colour and shape are becoming well understood (Ben Chaim *et al*., 2003; Thorup *et al*., 2000; Huh *et al*., 2001).

Thin : Morphologically the Capsicum fruit is a berry, sometimes with a few stone cells (sclerified inclusions in the fleshy portion) (Knapp, 2002). The pericarp consists of epidermal cells in regular order with a thick-grooved cuticle. Several rows of collenchymatously thickened beaded cells constitute the hypodermis. The mesocarp is formed by thick-walled headed cells; the inner mesophyll cells walled ground parenchyma and fibrovascular bundles. Giant cells (perhaps unique to Capsicum) occur on the inner wall of the endocarp (Fridvalsky and Nagy, 1966). The vascular bundles consist of xylem tissue with spiral vessels and phloem tissue. The pod has two, three or four locules, with each corresponding wall of the axile placenta having vesicles for production of capsaicinoids (Suzuki *et al*., 1980).

Usually there are many more flowers than fruits (Marcelis *et al*., 2004). The most obvious sign of assimilate competition or dominance among the organs is abscission of flowers and unripe fruits during the most active fruit-growth period, resulting in a cycling of flowering and fruit set (Hall, 1977; Clapham and Marsh, 1987; Bhatt and Srinivasa Rao, 1997; Marcelis *et al*., 2004). The most actively growing organ of a chili pepper plant after flowering is the fruit (Hall, 1977; Beese *et al*., 1982; Marcelis and Baan Hofman-Eijer, 1995). Fruit growth is dependent on ovule growth (whether fertilised). The fruit is ordinarily seeded, but

parthenocarpic forms exist (Heuvelink and Körner, 2001). The seed set affects development and subsequent growth of the fruit; on average there is a direct linear relationship between the number of seeds per fruit and final fruit size, until saturation at perhaps over 200 seeds per fruit (Marcelis and Baan Hofman-Eijer, 1997). The number of seeds per fruit ranged from 1 to 34 in wild northwestern Mexico populations of *C. annuum* (Hernández-Verdugo *et al*., 2001b). A low of 50-100 seeds per cultivated fruit (20-30% of maximum) is sufficient for maximal fruit set (Marcelis and Baan Hofman-Eijer, 1997); blocky sweet pepper (bell pepper) may average 150-300 seeds per fruit (Aloni *et al*., 1999).

The time from anthesis to a fully grown fruit varies considerably among different pod types (Bosland and Votava, 2000). Typically cultivated fruit reaches the mature green stage in 35-50 days after the flower is pollinated. This stage is horticulturally ripe for some uses, but still physiologically immature. Fruit maturity depends on the cultivar and the environmental conditions before and during maturation (Perry *et al*., 1993; Montes Hernández *et al*., 2004). The fruits are characterised as non-climacteric in ripening (Lownds *et al*., 1993), apparently lacking the typical increase in CO, and ethylene production as they ripen (Saltveit, 1977).

The fruits of most Capsicum are pungent, because the placenta accumulates capsaicinoids (e.g. capsaicin) (Zewdie and Bosland, 2001; Thompson *et al*., 2005), except in domesticated non-pungent (sweet) varieties which are mostly developed in *C. annuum* (Bosland and Votava, 2000). The pungency trait is controlled at a single locus chromosome 2; when the pungency gene Punl (also called C) is homozygous recessive (ie. present as pun1/pun1 or cc), the capacity to make capsaicinoids is lost (Stewart *et al*., 2005; Blum *et al*., 2002). In the pungent chili peppers, other genes variously affect the synthesis of capsaicinoids (Blum *et al*., 2003), and production is also affected by physiological interactions and the environment (Zewdie and Bosland, 2000a; Estrada *et al*., 2002; Sung *et al*., 2005). The individual fruit's pungency (content of capsaicinoids) is affected by its node position on the plant, whereas its capsaicinoid profile remains fairly constant (Zewdie and Bosland, 2000b; Estrada *et al*., 2002; Kirschbaum-Titze *et al*., 2002). Capsaicinoids increase with fruit growth to a maximum (e.g. 40-50 days after fruit set), then decline (Contreras-Padilla and Yahia, 1998). Capsaicinoids can be transported within the plant, with different capsaicinoid profiles found in stems and leaves (Estrada *et al*., 2002).

I.5.6 Fruit dispersal :

The red fruits of wild *C. annuum* var, glabriusculum attract birds, which eat them and disperse viable seeds, but their pungency discourages consumption by wild mammals (Vásquez-Dávila, 1996; Tewksbury *et al*., 1999; Tewksbury and Nabhan, 2001). Rats

experimentally fed hot chili peppers for 2-11 months became desensitised to aversion, but indifferent rather than developing a preference for this spicy food (Rozin *et al*., 1979). Nonetheless, the widespread and common little yellow-shouldered bat (Sturnira lilium), which sometimes favours solanaceous fruits (Passos *et al*., 2003; Galindo-González *et al*., 2000), has been reported to consume pungent Capsicum in northwestern Argentina and disperse the seeds which is favoured by local people who recognise this increases the number of wild plants, as they gather the fruits for home seasoning and village marketing (ludica, 1999),

I.5.7 Seed and germination:

The seed develops from a campylotropous ovule (Dharamadhaj and Prakash, 1978). Within a pod, the many seeds are attached to the placenta walls in close rows, mainly near the calyx end. The seeds are disk-like with a deep chalazal depression. The embryo is surrounded by a well-defined endosperm which makes up the bulk of food reserves for the embryo and young seedling. The endosperm lies directly in front of the radicle and consists of seven to nine thick cells (Watkins *et al*., 1985). *Capsicum annuum* seeds have mainly protein and lipids as storage reserves (Chen and Lott, 1992). The seed is covered by a parchment-like seed coat, which does not cause a mechanical restriction to germination (Watkins and Cantliffe, 1983b). Seed colour inheritance involves at minimum about three genes (Zewdie and Bosland, 2003). Seed size is somewhat dependent on the variety and growing conditions. Seed mass maturity may occur about 50 days after anthesis, with 10-12 more days required for maximum potential longevity but 17-21 days for maximal seedling dry weight (based on variation in time from sowing to emergence) (Demir and Ellis, 1992). An average *C. annuum* seed is about 5.3 mm long, 4.3 mm wide and 1 mm thick, with a surface area of 33 mm^2 (Chen and Lott, 1992).

Freshly harvested seeds of certain wild Capsicum species can exhibit dormancy (Bosland and Votava, 2000; Wien, 1997; IBPGR, 1983). An after-ripening period at room temperature may be required to remove dormancy (Randle and Homna, 1981). As C. anmam seeds age and lose viability (Ozcoban and Demir, 2002) they may become brown. Seed dormancy may be broken by treatment with 0.2 M. KNO, under white light (750-1250 lux) and alternating temperatures (30°/20°C or 30°/15°C) (cf. Hernández-Verdugo *et al*., 2001b). Seeds of cultivated C. anmum can be cryopreserved at -196°C and moisture content of 4.7-11.5%, and subjected to rapid or slow freezing and thawing (Quagliotti and Comino, 2003). Capsicum species seeds germinate well in a constant temperature range between 15°C and 30°C (Randle and Homna, 1980; cf. Dell'Aquila, 2004), and do not germinate when exposed to temperatures below 8°C or above 40°C (Choi, 1985). No special light requirements are necessary for

germination of domesticated chili pepper seeds, whereas seeds of wild *C. annuum* var. glabriusculum do not germinate in constant darkness (Hernández-Verdugo *et al*.; 2001b)

I.5.8 Stem:

Stem is densely branched and up to 60 cm (24 in) tall .

I.6 Sexual reproduction

I.6.1 Pollination

Capsicum species are usually self-compatible (Onus and Pickersgill, 2004), and *C. annuum* is a partially self-pollinating crop (Allard, 1960; Rylski, 1986); wind or similar mechanical disturbance may enhance self-pollination (Raw, 2000, Kristjansson and Rasmussen, 1991). Outcrossing is associated with insect pollinators, less with wind (Odland and Porter, 1941; Tanksley, 1984a; Raw, 2000). The proportion of plants cross-pollinated depends on several factors and can range from 2 to 90% (Pickersgill, 1997); in many localities, cross-pollination is predominant. The effect of outcrossing on fruit set of *C. annuum* is significant. Nagarathnam and Rajamani (1963) obtained only 6-11% fruit set when flowers were isolated to self-pollinate. Erwin (1937) found that 46% of self-pollinated flowers set fruit, compared to 71% for flowers left to open-pollinate by bee activity. In field research Capsicum should be considered facultative cross-pollinating species (Odland and Porter, 1941: Tanksley, 1984a). Breeders and seed producers thus need to undertake precautionary measures to prevent uncontrolled cross-pollination (Bosland, 1993). To produce ripe amounts of genetically pure seeds, seed certification programmes employ isolation as the control mechanism. Isolation requirements may range from 400 m for the Certified class to 1.6 km for the Foundation class (NMCIA, 1992) but depend on local conditions, for example being 300 m in Hungary but perhaps requiring 2-3 km or more in Australia (Derera *et al*., 2005).

The odourless flowers are visited by insects both for sugary nectar, which is mostly hexoses and low in daily amount (greatest on the day of anthesis), and also for their pollen (Rabinowitch *et al*., 1993; Vogel, 1998; Roldán-Serrano and Guerra-Sanz, 2004; Raw, 2000). Solitary bees, honeybees, bumblebees, aphids and thrips are likely to transfer the pollen grains, especially those that obtain pollen by buzz pollination, shaking the anthers (Andrews, 1995; Raw, 2000; de Ruijter *et al*., 1991; Kubišová and Háslbachová, 1991; Shipp *et al*., 1994; Dag and Kamer, 2001; Kristjansson and Rasmussen, 1991).

I.6.2 Crossability and hybridisation

Capsicum species do not hybridise with species in other genera of the Solanaceae (Berke, 2000). Pepper breeding continues to be highly rewarding for the improvement of Capsicum

(Poulos, 1994; Berke, 2000; Geleta and Labuschagne, 2004). Interspecific crossing between many Capsicum species has been tried experimentally (often repeatedly) for agronomic and taxonomic purposes (cf. Walsh and Hoot, 2001; Pickersgill, 1991, 1997; Onus and Pickersgill, 2004). Fertile hybridisations can occur between taxa within the *Capsicum annuum* complex to varying degrees (Jarret and Dang, 2004; Nwankiti, 1976; Kumar *et al*., 1987; Panda *et al*., 2004; Baral and Bosland, 2004), and also these species with C. baccatum but not with C. pubescens, Table 4 below gives a synopsis (cf. Yoon *et al*., 2004). Similar interspecific spontaneous or natural hybrids of these species are difficult to ascertain, but infrequently surmised (Jarret and Dang, 2004; Rodriguez *et al*., 1999). Their recognition is confounded by taxonomic uncertainty, the extensive variability from selection within the domesticated species for millennia to decades, and the plasticity of individual plants. Crossings between wild and semi-domesticated *C. annuum* var. glabriusculum, and between feral or weedy and domesticated *C. annuum* var, annuum, and these two complexes hybridising with each other, are probably a regular occurrence and vary in fertility (Jarret and Dang, 2004; Guzmán *et al*., 2005; Hernández-Verdugo *et al*., 2001a; Prince *et al*., 1992; Pickersgill, 1971). Crossing also is probable in many regions in the tropics between cultivated and feral C. frutescens (e.g. Yamamoto and Nawata, 2004, 2005; Symon, 1981; Wiggins and Porter, 1971).

I.6.3 Asexual reproduction

The chili pepper plant can be propagated asexually by means of cuttings and .34 grafting. Young cut shoots form whole independent plants with roots *in vitro* as well as in the field (Choi *et al*., 1999; Shirai and Hagimori, 2004). Scions from chili pepper plants graft successfully on stocks of chili pepper (Chung and Choi, 2002) as well as tomato (Deloire and Hébant, 1982). The grafted plants can set flowers and fruits. Capsicum grafting can induce genetic changes, which may provide variations of breeding value (Taller *et al*., 1998,1999).

I.7 Centres of origin and distribution:

I.7.1 Production at the global level :

The centre of diversity for Capsicum is in south-central South America (Eshbaugh, 1980; Hunziker, 1979; D'Arcy and Eshbaugh, 1974; Gonzalez and Bosland, 1991; WWF and IUCN, 1997), with the majority of species having some range in Brazil and/or Bolivia. Some of the non-domesticated species are gathered for occasional use. The primary centre of origin for domesticated *C. annuum* is in semi-tropical Mexico (Hernández-Verdugo *et al*., 1999, 2001a; Andrews, 1995; Long-Solís, 1998; Whitmore and Turner, 2002). The four other domesticated species are usually believed to have originated in South America (Eshbaugh *et al*., 1983; Walsh and Hoot, 2001; Denevan, 2001). The centres of origin and domestication of the other two

species in the *C. annuum* complex are not as clear (cf. Clement, 1999). Amazonia (in the northern area) is considered the centre for C. chinense ("habanero") (Velez, 1991; Toquica *et al*., 2003), and western Amazonia is perhaps the centre for C. frutescens ("tabasco"), which is **more morphologically variable** [**highly variable**] in Central America (Heiser, 1985; Hernández-Verdugo *et al*., 1999)**.** Bolivia is considered the centre of domestication for C. baccatum (aji) (in the subtropical east) and C. pubescens (rocoto) (in the mid-elevation Andes, where known only in cultivation) (Eshbaugh *et al*., 1983; Eshbaugh, 1993).

By molecular analysis, Loaiza-Figueroa *et al*. (1989) confirmed that the centre of domestication of *C. annuum* var. annuum, the cultivated variety, is the upland region (Sierra Madre Oriental) of central-eastern Mexico (in the states of Nuevo León, Tamaulipas, San Luis Potosí, Veracruz and Hidalgo). Its ancestor probably is the wild chiltepín (bird pepper), *C. annuum* var. glabriusculum (Dunal) Heiser & Pickersgill, which has a range of unclear natural extent from southern USA through Mesoamerica to Colombia and the Caribbean, and is sometimes wild-harvested still and semi-domesticated as well (Eshbaugh, 1993; Hernández-Verdugo *et al*., 2001a; Votava *et al*., 2002; Vásquez-Dávila, 1996; Guzmán *et al*., 2005).

The earliest archaeological evidence of Capsicum being used dates to 10,500 BP

(C. baccatum) and 10,000-9,500 BP (C. chinense) in the western Central Andes of Peru (Brack, 2003; Smith, 1980). Substantial evidence in Peruvian dry coastal river valleys, with specimens increasing from rare to moderately abundant through eight millennia, indicates expanding cultivation and domestication of Capsicum (Pearsall, 2003). Capsicum frutescens is recognised in the northwestern area by 4400-3200 BP (Brack, 2003). Intriguingly, Capsicum seems to be absent from the more recent Chiribaya culture of 1100-600 BP in far southern Peru (Flamini *et al*., 2003), even though earlier (2200-1400 BP) in southwestern Peru C. frutescens was being used. The importance of Capsicum is suggested by an obelisk from Chavín de Huantar (2800 BP) in the north-central Peruvian Andes, featuring a foundational Earth-crocodilian associated with (apparently) gourd, chili pepper, manioc (cassava) and peanut (Brotherston, 1979; Miller and Burger, 1995). Similarly, there is archaeological evidence from about 9000 BP for the use and subsequent domestication of *Capsicum annuum* in central-eastern and south-central Mexico in the states of Tamaulipas (near Ocampo), Puebla (Tehuacán Valley) and Oaxaca (Guilá Naquitz) (Pickersgill, 1984; Bosland, 1996; cf. Smith, 2001, 2005).

Capsicum was brought to Europe by Columbus in 1493 as the peppery spice that signified the success of his quest, and the early European voyagers to the Caribbean, Mesoamerica and South America encountered a plethora of variety and landraces of this common food as well as

medicinal plant (Sauer, 1966; Long-Solis, 1998). The ready appeal of Capsicum was such that within half a century it had been distributed as far as Asia, and it has heen integrated and continues to he diversified in cultures worldwide as it had been originally in the Americas (Ferrão, 1992; Andrews, 1992, 1995, 1998, 1999; DeWitt and Bosland, 1996; Eshbaugh, 1983; Yamamoto and Nawata, 2004, 2005).

The species of Capsicum were listed with their seemingly natural distributions by the International Board for Plant Genetic Resources (IBPGR, 1983) and updated by Eshbaugh (1993), Hernández-Verdugo *et al*. (1999) and Bosland and Votava (2000); they are listed in Table 2 as currently understood. There is modest uncertainty on the generic limits of Capsicum and more uncertainty on its tribal relatives (which at minimum include Lycianthes) (Eshbaugh, 1993; Hunziker, 2001; Knapp, 2002), and a lack of consensus on the number and in a few cases the botanical names of the known Capsicum species, and on the truly natural distributions of several species (rather than confounding naturalised with native populations).

Generally Capsicum spp. are grown as a spice in the equatorial regions of the countries like India, Mexico, United States of America, Japan, Turkey and African States (Sun, Y. L. *et al*., 2014; Masud Parvez, G. M., 2017). According to a data of 2017, the annual production of green chilli in Asian region was about 24 million tons from the cultivated lands of 1.3 million hectors . Around 68% of world's total green chilli was produced in Asian region and the highest five chilli producer countries were China, Turkey, Mexico, Indonesia and Spain. Due to the production of around 17 tons of chilli, China became the world's ripeest chilli producer and Mexico became the second ripeest with the production of 3.2 tons of chilli in 2017. After China and Pakistan, India is also considered as one of the ripeest chilli producers As shown in the Figure I.3 (Masud Parvez, G. M., 2017).

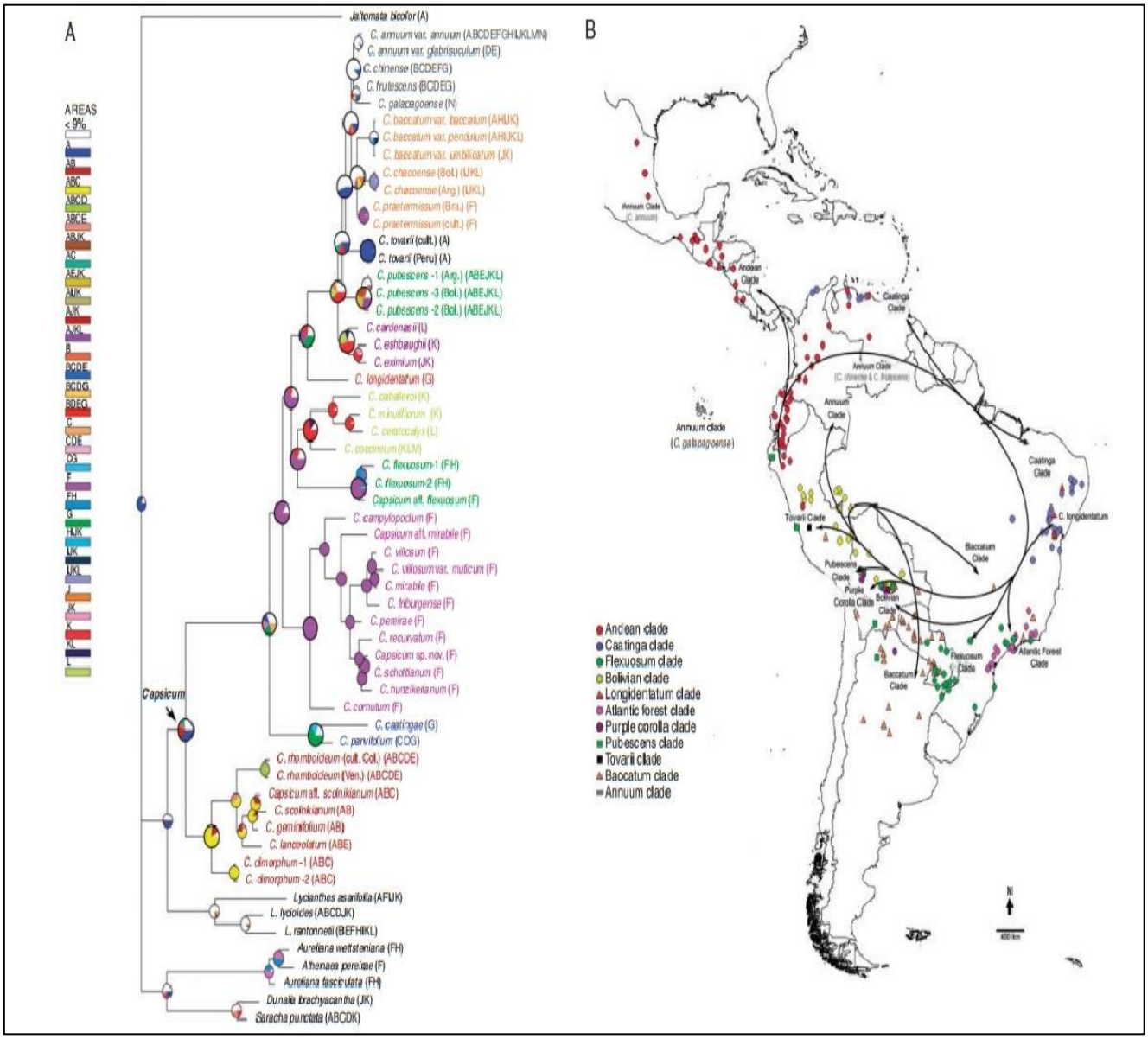

Figure I.3. Hypothesis of Capsicum expansion. (A) Ancestral areas reconstructed by Bayesian MCMC analysis. Pie charts are larger for the main nodes to make them more evident. Area assignment for each species is shown after taxon name. Colour codes reflect the major clades based on the phylogenetic results (grey scale for the Annuum Clade). References: A, Peru; B, Ecuador; C, Colombia; D, Venezuela; E, Central America (including Mexico); F, south-eastern Brazil (Atlantic Forest ecoregion); G, central–eastern Brazil (Caatinga ecoregion); H, north-eastern Argentina and eastern Paraguay (Ako Parana Atlantic forestsecoregion); I, central–western Paraguay; J, north-western and central Argentina; K, northern, north-eastern and south-eastern Bolivia (mostly lowlands); L, western and south-western Bolivia (mostly highlands M, western Brazil; and N, Galapagos Islands. (B) Schematic expansion of the species. The arrows represent clades and monotypic lineages going across and/or pointing to the areas inhabited by their species Markings in different colours/shapes indicate selected population localities. In

order not to over-complicate the presentation, the taxa of the Annuum Clade are mentioned in their appropriate place but without markings and partly without arrows

I.7.2 Morphological characters for identification:

The five domesticated species are differentiated by using morphological characters that rely primarily on colour and morphology of flowers and seeds (Andrews, 1995; DeWitt and Bosland, 1996), as shown in Table 3. However, identifying some plants in the diverse *C. annuum* complex can be problematic (Pickersgill *et al*., 1979; Bosland and Votava, 2000; Jarret and Dang, 2004; Baral and Bosland, 2004). Capsaicinoid profiles are not reliable unique indicators for identification, though the profile may be useful as a supplementary character. In one study (7-58 accessions per species), the accuracy of identification based solely on capsaicinoid profiles, in the *C. annuum* complex was 82% of the C. chinense accessions, 57% for *C. annuum* and just 20% for C. frutescens (but its sample was only 10 accessions); and similarly was 59% for C. baccatum, and 86% for the distinctive C. pubescens As shown in the table I.3 (Zewdie and Bosland, 2001).

Table I.3. Morphological characters that generally differentiate the domesticated species of Capsicum.

Species	Flowers per node	Calyx	Corolla	Corolla-lobe basal spots	Anther colour	Seed colour
C. annuum var, annuum	1(5-)	no ring: often teeth	white to dingy white (rarely purple)	none	blue-purple	straw (tan)
C. frutescens	usually 2-4(1-6)	no ring usually no teeth	greenish white or greenish	none	blue-purple	straw (tan)
C. chinense	(1-) 2 (-5)	annular ring: no teeth	greenish white or white	none	Blue	straw (tan)
C. baccatum var. pendulum	(-2)1	no ring: teeth	white (cream) or greenish-white	yellow-green	white to yellowish	straw (tan)
C. pubescens	1	no ring: teeth	purple or purple-white	none	purple (purple-white)	black (brown/black)

Source: after Lippert *et al*, 1966; Heiser, 1985; Greenleaf, 1986; Eshbaugh, 1993, Jarret and Dang, 2004

I.7.3 Production in Algeria :

Capsicum annuum is one of the five domesticated species of Capsinew genus. Because of its high commercial advantage, it has been popular and of great interest since antiquity (Padilha *et al*., 2016; Barboza *et al*., 2019). The consumption of pepper occurs throughout the world in various ethnic groups, adding flavor, aroma, and color to food (Ornelas-Paz *et al*., 2013) fresh consumption, dry powder and pasta. It is grown across the whole country in a different of settings varying from unripe-scale subsistence farming to ripe-scale commercial enterprises. Nevertheless, there are a few records on hot pepper landraces acreages and yield, which do not accurately reflect the crop's current economic importance. In Algeria, 2 400.1 hectares are annually cultivated with chili peppers and total production is estimated at 174 234.1 tons. Yield tends to vary considerably depending on numerous factors mainly the origin of hot pepper variety planted which is closely related to the crop system. In Biskra province for example, yield records ranging from Pepper is an unavoidable component of the Algerian population's dict. It is consumed in various forms including El Oued is one of Algeria's most densely populated Wilayas in the Sahara The agriculture sector employs 42% of the Wilaya's active workforce. Available groundwater resources in this agricultural region are estimated at 4.9 billion cubic metres The agricultural sector is currently booming in the Wilaya, where the total agricultural area is around hectares, or 35.70% of the wilaya's total area. The useful agricultural area covers around 52911 hectares, or 3.32% of the total agricultural area, of which 51456 hectares are 1591752 irrigated As shown in the Figure I.4.

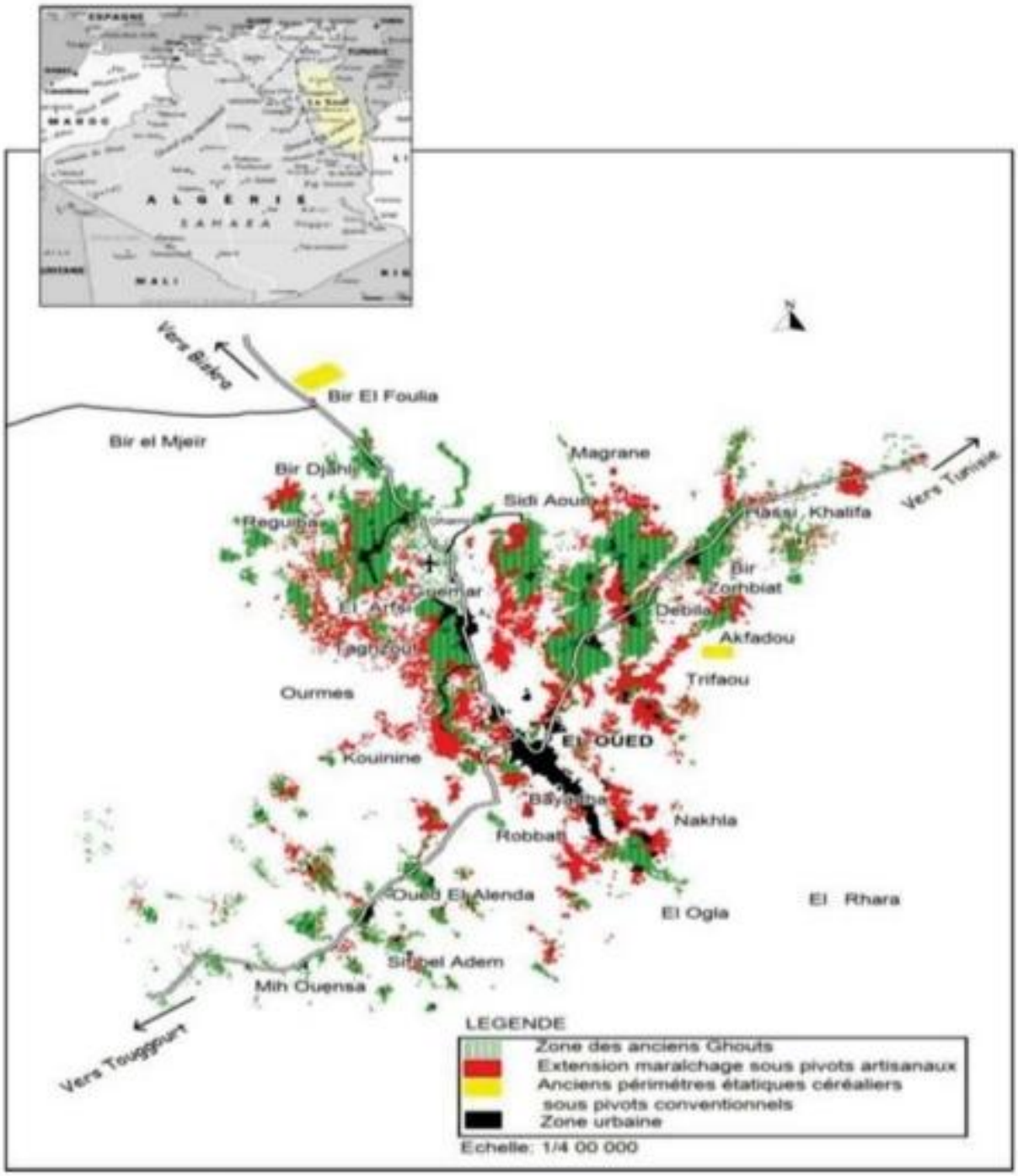

Figure I.4 : Municipalities of the Oued Souf region (INSID, 2011)

Capsicum annuum: Hot pepper is an unavoidable component of the Algerian population's diet. It is consumed in various forms including fresh consumption, dry powder and pasta. It is grown across the whole country in a different of settings varying from unripe-scale subsistence farming to ripe-scale commercial enterprises. Currently, the Southeast Algeria, including Ghouts, represent the last rampart of the cultivation of pepper landraces. Over many centuries, farmers have cultivated pepper crops using traditional practices to preserve local populations in various environments, saving and exchanging the seeds, they produced and transferring them

from one generation to the next promoting the occurrence of both outcrossing and introgression. This obviously led to phenotypically different populations. Usually, local varieties have varying names that carry the meaning of a specific region or characteristic or use. However, despite the important cultivated area, all pepper landraces in Algeria have the same appellation: Arabian pepper or "Felfel Arbi". Peppers are grown on an area of 119 ha, producing 42700 qx, or a yield of 358.8 quintals per hectare. It is grown in Ghouts where there are wells.

Chillies are grown on 630 hectares, with an annual production of 173,080 qx and a yield of 274.9 qx/ha. It is grown in open fields and greenhouses. The chillies of Ghouts are generally grown from local seeds, known as "Laarbi." As shown in the table I.4.

Table I.4: List of the hot pepper accessions collected in Algeria

Codes	Localities	Provinces
BD1	Doucen	Biskra
BD2		
BD3		
BDj	Djemourah	
BMg	El Megsem	
BSO	Sidi Okba	
BZ1	Zeribet El Oued	
BZ2		
EHk	Hassi Khelifa	El Oued
EL1	Lizireg	
EL2		
EL3		
EL4		
EL5		
ETr	Trifaoui	
MAk	Ain AlKhadra	Msila
MEk	El-Khoubana	
MOa	Ouled Ammar	
MOi	Ouled Ichir	
TFe	Ferkane	Tebessa
TGr	Touggourt	Touggourt

I.8 CROP PRODUCTION AND USE

Chili peppers are grown worldwide, either outside in fields or in greenhouses. The ability to produce a quality crop in such a wide range of climates and conditions has helped to make chili pepper a globally common crop. Because of the extensive cultivation, adaptation and

variability of *C. annuum*, it is difficult to generalise to what is typical, and there is no single method for production (Bosland and Votava. 2000).

I.8.1 Environmental conditions :

Chili pepper is a warm-season crop (Rodriguez-Rey *et al*., 2000), and highly susceptible to frost. Watkins and Cantliffe (1983a) showed that at 25°C radicle emergence required 3.5 days, whereas at 15°C, 9 days were required. Seedling emergence from a soil depth of 1.2 cm took 8-9 days at temperatures from 25-35°C (Lorenz and Maynard, 1980), but was prevented below 15°C (Wien, 1997). The leaf unfolding rate of seedlings (based on maximum leaf count), which is also a measure of node and internode formation, was optimal at an average daily temperature of 26°C (Si and Heins. 1996).

The plant is usually indeterminate and has continuous sympodial branching, with the individual branch systems apparently functioning as relatively autonomous integrated physiological units (Thomas and Watson, 1988; cf. de Swart *et al*., 2004). For a high yield of good quality fruit, Bakker and van Uffelen (1988) found that mean air temperatures of 21-23°C were optimal during vegetative growth, followed by 21°C during fruit growth. The minimum temperature for growth and development is 18°C, below which growth is trivial, with plants in the 5-15°C range growing poorly (Sanders *et al*., 1980). The most growth in the vegetative stage occurs at 25-27°C day temperature and 18-20°C night temperature (Dorland and Went, 1947; Bakker and van Uffelen, 1988). Day temperature lower than night temperature is detrimental to vegetative growth (as is a low night temperature of 12°C). Nonetheless, to grow compact greenhouse seedlings, higher night temperature is preferable (Si and Heins, 1996; Sysoyeva and Kharkina, 2000).

Maximum flower set occurs when day and night temperatures are between 21°C and 16°C. Flowers drop when the night temperature is above 24°C. Yields are high when the daily air temperature during fruit set ranges between 18-32°C. Fruits do not set when the mean daily temperature is above 32°C. or is below 16°C or when cooler, the fruits are malformed (Olareweju, 1988; Aloni *et al*., 1999). Productivity is constrained by the adverse effects of high temperature on fruit set, and the detrimental influence of low temperature on fruit shape (Rylski and Spigelman, 1982; Rylski, 1973).

I.8.2 Agricultural practices :

The ideal soil for producing chili pepper is deep, well-drained, the intermediate maturity stage -textured sandy loam or loam that holds moisture and has some organic matter. Plants can be started by direct seeding, or by transplanting after initial growth in trays (Bosland and

Votava, 2000); the plants are started in greenhouses or hotbeds in many production areas, or in outdoor seedbeds in mild-climate areas. Chili pepper plants are transplanted when they are 6-8 weeks old. Prior to field planting, the plants should be hardened but not excessively.

Whether the field population is established by transplanting or direct seeded, the optimum crop is dependent upon row spacing and between-row spacing of the plants, and the type grown (Bosland andVotava, 2000). Chili peppers require adequate amounts of most major and minor nutrients; the most-utilised are normally N and P. Plastic mulch maintains moisture in the soil; increases soil temperature and early yields; reduces weed populations, fertiliser leaching and soil compaction; and protects fruits from soil deposits and soil micro-organisms. Competition between weeds and chili peppers for nutrients, light and water is a serious problem in production (Lee and Schroeder, 1995). A successful weed control programme is essential for producing a healthy crop. Abiotic stresses include extreme temperatures, moistures, light, nutrients, pH, pollutants and pesticides.

Row covers or tunnel planting systems have been used for production in the field because of their effectiveness to alter microclimates. Chili pepper is sensitive to excessive water (Suh *et al*., 1987). Irrigation is not necessary in areas with regular and ample rain, although it generally is essential in arid and semi-arid regions. Chili pepper is a shallow-rooted crop (González-Cervantes *et al*., 2004). The amount and frequency of irrigation depend on soil type, bed type, plant size, humidity, wind, sunlight and prevailing temperatures. A limited supply of water during the rapid vegetative-growth period reduces the final yield (Beese *et al*., 1982; Srinivasa-Rao and Bhatt, 1988; Sato *et al*., 2003). Fruits grown under water deficit may have a higher concentration of capsaicin (Sung *et al*., 2005).

Chili pepper plants can be made to behave perennially under greenhouse conditions, with environmental control carried out by air temperature regulation, supplemental light, and CO, enhancement as well. Regular removal of flowers leads to faster vegetative growth (Hall, 1977; Clapham and Marsh, 1987). In The Netherlands non-pungent chili peppers are greenhouse-grown on 1200 ha, and about a third of the workers develop an allergy to the pollen, which can be alleviated by introducing honeybees to remove pollen (Blacquière *et al*., 2004).

I.8.3 Biotic stresses :

Production can be diminished by various biotic stresses. Chili pepper is susceptible to diseases and pests that can be primary constraints on cultivation (Bosland and Votava, 2000; DeWitt and Bosland, 1993), and their control is one of the most important factors in producing a profitable crop. The diseases and pests usually reduce both quality and quantity of fruits

Diseases from bacteria infecting the chili pepper plants include bacterial spot (Xanthomonas campestris pv. vesicatoria), bacterial canker (Corynebacterium michiganense or Clavibacter michiganensis subsp. michiganensis), bacterial soft rot (Erwinia carotovora pv. carotovora) and bacterial wilt (Pseudomonas solanacearum or Ralstonia solanaceraum). The plants are susceptible to fungi which cause diseases such as anthracnose (Colletotrichum spp.), early blight (Alternaria solani), Cercospora leaf spot (Cercospora capsici), damping-off/seedling disease (Pythium, Rhizoctonia, Fusarium, etc.), Fusarium stem rot (Fusarium solani), grey mold (Botrytis cinerea), Phytophthora blight and root rot (Phytophthora capsici), powdery mildew (Leveillula taurica ot Oidiopsis taurica), Rhizoctonia root rot (Rhizoctonia solani), Stemphylium leaf spot (Stemphylium botryosum f. sp. capsicum), gray leaf spot (Stemphylium solani and S. lycopersici), southern blight (Sclerotium rolfsii), Verticillium wilt (Verticillium dahliae) and white mold (Sclerotinia sclerotirum). Among the many viruses affecting chili peppers are alfalfa mosaic alfamovirus (AMV), cucumber mosaic cucumovirus (CMV), beet western yellows luteovirus (BWYV), pepper mottle potyvirus (PepMoV), pepper veinal mottle potyvirus (PepVMoV), potato potyvirus Y (PVY), tobacco etch potyvirus (TEV), pepper mild mottle tobamovirus (PepMMoV), pepper ringspot tobravirus (PepRSV), tomato spotted wilt tospovirus (TSWV), pepper golden mosaic bigeminivirus (PepGMV), pepper Huasteco bigeminivirus (PHV or PepHV). Texas pepper bigeminivirus (TPV) and beet curly top hybrigeminivirus (BCTV).

Production is affected by many insect pests such as cutworms, grubs (Phyllophaga spp.), flea beetles (Epitrix spp.), hornworms (Manduca sexta and M. quinquemaculata), grasshoppers, leafminers ,fruit worms (Heliothis assulta and H. zea, Spodoptera spp. armyworms, etc.). European corn borer (Ostrinia nubilalis), green peach aphid (Myzus persicae), melon or cotton aphid (Aphis gossypii), leafhoppers, stink bugs, tarnished plant bug (Lygus lineolaris), thrips, whiteflies, chili weevil (Anthonomus eugenii) and chili pepper maggot (Zonosemata electa), and by spider mites (Tetranychus spp.) and nematodes Chili pepper production is also influenced by physiological disorders such as flower-bud abscission and flower abscission, blossom-end rot, sunscald, abnormal fruit shape, colour spotting, and fruit cracking.

I.8.4 Experience and world statistics :

Chili pepper is harvested at different fruit stages, depending on the final use. Fresh chili pods often are harvested at a physiologically immature (but horticulturally mature) stage. The dehydrated and. mash industries use physiologically mature fruits, generally showing red colour.

Chili pungency is measured by determining the capsaicinoids content of the fruit, which can be accomplished by several industrial (laboratory) procedures, and as well by a subjective dilution-and-detection test ("taste test") scored as Scoville Heat Units (Scoville, 1912; Korel *et al*., 2002; Bosland and Votava, 2000; Krishna De, 2003; Reilly *et al*., 2001). Physiologically, capsaicinoids cause the heat sensation by activating and then desensitising certain sensory nerve fibres, which is mediated via a receptor (VR1) in the pain pathway (Caterina and Julius, 2001; Bhave *et al*., 2002). Culinary or medicinal results can be favourable (Rozin, 1990; Palevitch and Craker, 1995), whereas exposure to excessive amounts can range from avoidance behaviour to severe toxicity (Krenzelok and Provost, 1995).

The production of chili pepper for spice, vegetable, and other uses increases every year. It is estimated that it is annually cultivated on more than 1.5 million hectares, in numerous countries (FAO, 2003). Forty-six percent of production is in Asia (with China the principal producing country). Southern Europe is the second most important producing region, with 24% of world production. The countries with harvest area of more than 70,000 ha are China, India, Indonesia, Mexico, Korea, Nigeria, Ghana and Turkey. As shown in the Figure I.5.

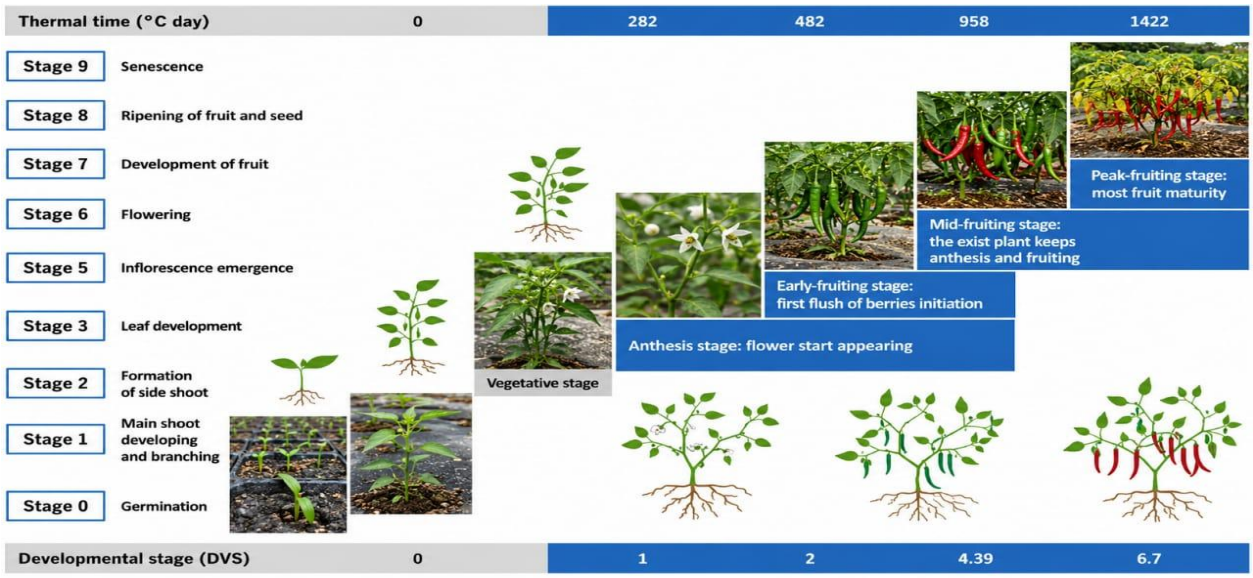

Figure I.5: Illustrative phenological growth pattern of chili pepper.

The key developmental stages of WOFOST-Chili are displayed on the horizontal axis at the bottom, with the corresponding temperature sums at the top. On the vertical axis, the growth of chili pepper is described, using the Biologische Bundesanstalt, Bundessortenamt and CHemical industry scale.

I.9. Chemical composition

Peppers contain a wide range of bioactive compounds such as capsaicinoids, polyphenols, flavonoids, and ascorbic acid, which are responsible for their nutritional and functional properties (Manikharda *et al*., 2018; Choi *et al*., 2022). These compounds significantly influence the physicochemical characteristics and antioxidant activity of peppers (Howard *et al*., 2000; Choi *et al*., 2022). Capsaicinoids are the main compounds responsible for the pungent taste of peppers (Kim *et al*., 2002; Srinivasan, 2016), while polyphenols and flavonoids contribute to their antioxidant potential and health-promoting effects (Choi *et al*., 2022).

I.9.1 Phenolic Compounds

Phenolic compounds are a ripe group of secondary plant metabolites widely distributed in fruits, vegetables, and medicinal plants. They are characterized by the presence of one or more aromatic rings bearing hydroxyl groups. Recent studies (2020–2024) have demonstrated that these compounds exhibit strong biological activities, particularly antioxidant, anti-inflammatory, and free radical scavenging effects, which make them important in preventing oxidative stress-related diseases (Tomas-Barberan & Andrés-Lacueva, 2021; Shahidi & Ambigaipalan, 2021).

Phenolic compounds are generally classified into phenolic acids, flavonoids, and related polyphenolic structures. Among them, flavonoids such as quercetin and luteolin, and phenolic acids such as caffeic acid, are among the most studied due to their high antioxidant potential (Agati *et al*., 2020; Rasines-Perea & Teissedre, 2020). Carotenoids, although not phenolic by structure, are often discussed alongside them because of their complementary antioxidant roles in biological systems (Rodríguez-Concepción & Avalos, 2022).

I.9.1.1 Phenolic acids

Phenolic acids (e.g., caffeic acid, ferulic acid) are known for their strong radical scavenging ability and protective effects against oxidative damage (Rasines-Perea & Teissedre, 2020).

I.9.1.2 Flavonoids

Flavonoids (e.g., quercetin, luteolin) are powerful antioxidants that also show anti-inflammatory, cardioprotective, and anticancer activities (Agati *et al*., 2020; Dabeek & Marra, 2021).

I.9.1.3 Carotenoids (optional)

Carotenoids act mainly as protective pigments with antioxidant properties that reduce oxidative stress in biological systems, particularly by inhibiting lipid peroxidation As shown in the table I.5 (Rodríguez-Concepción & Avalos, 2022).

Table I.5: Major phenolic compounds identified in *Capsicum annuum L*. and their biological activities

Phenolic compound	Type	Biological function
Quercetin	Flavonoid	Strong antioxidant, anti-inflammatory, free radical scavenger
Luteolin	Flavonoid	Anti-inflammatory, neuroprotective, antioxidant
Caffeic acid	Phenolic acid	Free radical scavenger, anti-oxidative protec
ferulic acid	Phenolic acid	Antioxidant, protects cells from oxidative stress
β-carotene	Carotenoid	Antioxidant, protects against lipid peroxidation

I.9.2 Capsaicinoids

Capsaicinoids are a group of alkaloid secondary metabolites mainly found in fruits of the genus Capsicum. They are responsible for the characteristic pungency (spicy heat) of peppers. These compounds are chemically characterized as amides formed between vanillylamine and different fatty acid chains. Recent studies (2020–2024) have demonstrated that capsaicinoids exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, analgesic, antimicrobial, and potential therapeutic effects, making them important compounds in both food science and pharmaceutical applications (Alonso-Villegas *et al*., 2023; Srinivasan, 2020).

Capsaicinoids are generally composed of several related compounds, mainly capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. Among them, capsaicin and dihydrocapsaicin are the most abundant and are primarily responsible for the pungency of peppers, while nordihydrocapsaicin is present in unriper amounts but still contributes to biological activity. These compounds also play an important role in oxidative stress reduction and show significant medicinal potential.

I.9.2.1 Capsaicin

Capsaicin is the major capsaicinoid responsible for the pungent sensation in peppers. It is widely studied for its ability to activate TRPV1 receptors, which are involved in pain and heat

perception. It also exhibits analgesic and anti-inflammatory properties (Nilius & Appendino, 2020).

I.9.2.2 Dihydrocapsaicin

Dihydrocapsaicin is structurally similar to capsaicin and contributes significantly to the overall pungency of peppers. It also shows strong antioxidant activity and has been associated with anti-inflammatory and metabolic regulatory effects (Luo *et al*., 2021).

I.9.2.3 Nordihydrocapsaicin

Nordihydrocapsaicin is a minor capsaicinoid found in lower concentrations. Despite its limited abundance, it contributes to the overall pungency and exhibits antioxidant and biological activities similar to other capsaicinoids As shown in the table I.6 (Alonso-Villegas *et al*., 2023).

Table I.6: Major capsaicinoids identified in *Capsicum annuum L*. and their biological activities

Capsaicinoid	Type	Biological function
Capsaicin	Major capsaicinoid	Responsible for pungency; analgesic and anti-inflammatory via TRPV1 receptor activation
Dihydrocapsaicin	Major capsaicinoid	Contributes to pungency; antioxidant and anti-inflammatory activity
Nordihydrocapsaicin	Minor capsaicinoid	Supports pungency; antioxidant and bioactive properties

I.9.3 Vitamins and Minerals

Vitamins and minerals are essential micronutrients present in *Capsicum annuum L*. that contribute significantly to its nutritional and functional value. Peppers are particularly rich in vitamin C, vitamin A (as provitamin A carotenoids), and vitamin E, along with important minerals such as potassium, magnesium, and iron. Recent studies (2020–2024) have shown that these nutrients play a key role in enhancing antioxidant defense systems, supporting immune function, and reducing the risk of chronic diseases associated with oxidative stress (Choi *et al*., 2021; Llorent-Martínez *et al*., 2020).

Vitamins in *Capsicum annuum L*. are mainly responsible for antioxidant protection and metabolic regulation, while minerals contribute to physiological and biochemical processes such as blood pressure regulation, enzyme activation, and oxygen transport. These nutrients act

synergistically with phenolic compounds and capsaicinoids, enhancing the overall health benefits of peppers As shown in the table I.7:

Table I.7: Major vitamins and minerals identified in *Capsicum annuum L*. and their biological activities

Nutrient	Type	Biological function
Vitamin C	Vitamin	Strong antioxidant, immune system support, collagen synthesis
Vitamin A	Vitamin	Vision health, cellular growth, antioxidant activity
Vitamin E	Vitamin	Protects cell membranes from oxidative stress
Potassium	Mineral	Blood pressure regulation and fluid balance
Magnesium	Mineral	Enzyme activation, muscle and nerve function
Iron	Mineral	Oxygen transport and hemoglobin formation

I.10 Nutritional value of *Capsicum annuum L.*

I.10.1 Nutritional contribution

Capsicum annuum L. is considered one of the most important vegetable crops due to its high nutritional value and rich composition of bioactive compounds. Peppers are characterized by low caloric content and high levels of vitamins, minerals, dietary fibers, and antioxidant compounds, making them an important component of a healthy diet. They are particularly rich in vitamin C, provitamin A carotenoids, phenolic compounds, flavonoids, and capsaicinoids, which contribute significantly to their nutritional and functional properties (Alonso-Villegas *et al*., 2023; Younes & Mustafa, 2024). In addition, peppers contain essential minerals such as potassium, magnesium, iron, zinc, and calcium, which play important physiological roles in the human body (Stoleru *et al*., 2023).

Recent studies have shown that the nutritional composition of *Capsicum annuum* varies according to cultivar, ripening stage, environmental conditions, and agricultural practices. Red peppers generally contain higher concentrations of carotenoids and vitamin C than green peppers due to the accumulation of pigments during ripening (Alonso-Villegas *et al*., 2023). Moreover, sustainable fertilization and biological treatments were reported to improve the nutritional quality and phytochemical content of sweet peppers by increasing mineral concentration, phenolic compounds, and antioxidant activity (Stoleru *et al*., 2023; Tahmasebi *et al*., 2023).

Peppers also contribute nutritionally through their high antioxidant capacity. Phenolic compounds, flavonoids, carotenoids, and ascorbic acid act as natural antioxidants that protect

cells from oxidative stress and free radical damage (Ali *et al*., 2025). Due to these characteristics, *Capsicum annuum* is increasingly recognized as a functional food with both nutritional and health-promoting properties As shown in the table I.8 (Younes & Mustafa, 2024)

Table I.8: Nutritional composition of *Capsicum annuum L*. Adapted from Téllez-Pérez *et al*. (2012).

	Sec /100g	Frais/100g	% Apport
Energie (kcal)	280–350	20–40	
Protéines (g)	11–18	0.8–2.0	
Lipides (g)	3–16	0.2–0.6	
Cendres (g)	6–8	0.4–0.9	
Hydrates de Carbone (g)	51–70	5.4–9.5	
Fibres totaux (g)	21–29	0.9–3.7	
Glucides (g)	38–41	2.0–5.3	
Minéraux			
Calcium (mg)	45–134	7–18	**0.7–1.8**
Fer (mg)	6–11	0.3–1.2	**2.7–8**
Magnésium (mg)	88–188	10–25	**3.2–8.1**
Phosphore (mg)	159–327	20–46	**2.9–6.6**
Potassium (mg)	1870–3170	175–340	**8.75–17**
Sodium (mg)	43–193	1–13	**0.04–0.54**
Zinc (mg)	1–2	0.1–0.3	**1.1–2.5**
Cuivre (mg)	0.2–1.4	0.07–0.13	**4.4–8.9**
Manganèse (mg)	0.8–1.9	0.10–0.24	**4.5–10.8**
Sélénium (µg)	2.9–3.7	0.1–2	**0.2–3.6**
Vitamines			
Vitamin C (mg)	2–31.4	44.3–183.5	**73–306**
Vitamine B1 (mg)	0.1–1.2	0.03–0.14	**2.5–13.1**
Vitamine B2 (mg)	1.2–2.4	0.03–0.09	**2.5–8.2**
Vitamine B3 (mg)	6.4–7.4	0.05–1.2	**0.3–8.9**
Vitamine B5 (mg)	0.5–2	0.10–0.32	**2.0–6.3**
Vitamine B6 (mg)	0.8–.5	0.22–0.51	**17.2–39.1**
Vitamine B9 (µg)	51–229	10–47	–
Vitamine A (µg)	1020–3860	10–157	**1–15.7**
Vitamine E (mg)	3–4	0.37–0.69	**4.6–8.6**
Vitamine K (µg)	108–114	4.9–14.3	**8.9–26**

I.10.2 Health importance

The health importance of *Capsicum annuum L*. is mainly associated with its high content of antioxidant and bioactive compounds, including capsaicinoids, phenolic acids, flavonoids, carotenoids, and vitamins A, C, and E. These compounds exhibit significant antioxidant, anti-inflammatory, antimicrobial, anticancer, and cardioprotective properties (Mandal *et al*., 2023). Regular consumption of peppers has been linked to the reduction of oxidative stress and inflammation, which are major factors involved in chronic diseases such as cardiovascular disorders, diabetes, obesity, and cancer (Ali *et al*., 2025).

Capsaicin, the major capsaicinoid found in hot peppers, has attracted considerable scientific interest because of its pharmacological activities. Studies have demonstrated that capsaicin can modulate inflammatory pathways, improve lipid metabolism, and contribute to body weight regulation and cardiovascular protection (Mandal *et al*., 2023). Furthermore, carotenoids and vitamin C found in peppers play an important role in protecting cells against lipid peroxidation and oxidative damage, thereby supporting immune function and reducing the risk of degenerative diseases (Younes & Mustafa, 2024).

Phenolic compounds and flavonoids present in peppers also contribute to their health-promoting effects through their ability to neutralize reactive oxygen species and inhibit inflammatory mediators. These compounds have been associated with neuroprotective, antimicrobial, and anticancer activities (Sanatombi, 2023). Consequently, *Capsicum annuum* is increasingly considered a valuable functional food and a promising natural source of bioactive compounds for disease prevention and health promotion As shown in the table I.9:

Table I.9: Major health benefits of *Capsicum annuum L*. Adapted from Mandal *et al*. (2023), Sanatombi (2023), and Ali *et al*. (2025).

Bioactive compound	Health importance
Capsaicin	Anti-inflammatory, anti-obesity, cardioprotective
Vitamin C	Immune support and antioxidant activity
Carotenoids	Protection against oxidative stress and lipid peroxidation
Phenolic compounds	Free radical scavenging and disease prevention
Flavonoids	Anti-inflammatory and anticancer properties
Capsanthin	Antioxidant and protective pigment activity

I.11 Preservation and Processing

I.11.1 Dehydration (Drying)

Drying is a key preservation technique used to extend the shelf life of *Capsicum annuum L*. by reducing moisture content, thereby inhibiting microbial growth and enzymatic reactions that cause spoilage. Modern studies show that advanced drying methods such as microwave drying, vacuum drying, and freeze-drying significantly improve the retention of color, antioxidants, and bioactive compounds in chili peppers (Elmatsani *et al*., 2024; Tiamiyu *et al*., 2023).

Pre-treatments such as blanching, ultrasound, and hot-air impingement have also been reported to enhance drying efficiency and preserve important compounds such as capsaicinoids and phenolics (Elmatsani *et al*., 2024). In addition, postharvest treatments like irradiation are

increasingly applied to improve microbial safety in dried chili products while maintaining sensory quality (Balakrishnan *et al*., 2021).

I.11.2 Freezing

Freezing is widely applied for the preservation of fresh *Capsicum annuum* due to its high water content. However, improper freezing conditions may lead to ice crystal formation, causing cellular damage and quality deterioration in texture, color, and nutritional composition.

Recent research indicates that rapid freezing techniques, particularly Individual Quick Freezing (IQF), significantly reduce tissue damage and better preserve physicochemical properties compared to conventional freezing methods (Tiamiyu *et al*., 2023). Moreover, optimized freezing conditions have been shown to extend shelf life while maintaining the overall quality of bell peppers during storage (Sanatombi & Rajkumari, 2020).

I.11.3 Brining (Pickling)

Brining is an important preservation method based on acid diffusion into the fruit tissue until a target pH (≤ 4.6) is reached, which inhibits microbial growth and ensures product stability.

Recent studies highlight that traditional brining processes are being improved by reducing chemical preservatives and adopting controlled fermentation techniques. This shift is mainly driven by health concerns related to additives such as sodium bisulfite and the growing demand for natural preservation methods (Sanatombi & Rajkumari, 2020; Food Reviews International, 2021–2024).

Furthermore, the use of natural antimicrobials and organic acids is increasingly replacing synthetic additives, while still ensuring safety and extending the shelf life of pepper-based products (Tiamiyu *et al*., 2023).

I.12 Pepper Diseases (*Capsicum annuum L.*)

I.12.1 Fungal Diseases

Fungal diseases are among the most important threats affecting *Capsicum annuum L*., including Fusarium wilt . These diseases cause significant losses in both yield and fruit quality, particularly under warm and humid environmental conditions.

Recent reviews indicate that fungi such as Phytophthora capsici, Fusarium spp., and Rhizoctonia solani are responsible for severe diseases leading to plant wilting as well as root and fruit rot. These pathogens are difficult to control using conventional chemical fungicides

due to their persistence in soil and environmental adaptability (Sanogo *et al*., 2023; Tiamiyu *et al*., 2023).

Furthermore, fungal diseases are considered among the most destructive constraints in pepper production worldwide, with more than 58 pathogenic fungal species reported to infect Capsicum crops, leading to substantial agricultural losses (Khasanov *et al*., 2021).

Figure I.6: Fusarium Wilt of Pepper (*Capsicum annuum L*.) Caused by Fusarium oxysporum

I.12.2 Bacterial Diseases

Bacterial diseases represent a major constraint in pepper cultivation, including bacterial spot. These diseases result in reduced productivity and severe damage to leaves and fruits.

These pathogens are commonly transmitted through contaminated soil, water, and infected seeds, making their management particularly challenging in humid agricultural environments.

Recent studies highlight that Integrated Disease Management (IDM), including good agricultural practices, use of resistant varieties, and biological control approaches, is the most effective strategy for reducing bacterial disease incidence in pepper crops (Khasanov *et al*., 2021; Tiamiyu *et al*., 2023).

Figure I.7: Bacterial Spot of Pepper (*Capsicum annuum L.*) Caused by Xanthomonas spp.

I.12.3 Viral Diseases

Viral diseases are among the most serious threats to pepper production due to their rapid spread and the lack of curative treatments. These diseases cause severe growth reduction, leaf deformation, and significant yield losses.

-The most important viruses affecting pepper include:

*Pepper mild mottle virus (PMMoV)

Recent reviews identify PMMoV as one of the most destructive viruses affecting pepper crops globally. It belongs to the genus Tobamovirus and infects different Capsicum species, leading to major economic losses in production (Kumari *et al*., 2023).

Moreover, plant viral diseases in pepper are considered a major global constraint to productivity and are primarily transmitted through insect vectors such as whiteflies and aphids, as well as through infected seeds (Khasanov *et al*., 2021; Tiamiyu *et al*., 2023).

Figure I.8 : Pepper Mild Mottle Virus (PMMoV) Infection in *Capsicum annuum L.*

I.13 Uses and Applications of *Capsicum annuum L.*

I.13.1 Agro-food and Industrial Use

Recent studies indicate that *Capsicum annuum L.* (sweet and hot pepper) is one of the most important agricultural crops widely used in the food industry due to its rich content of bioactive compounds such as carotenoids, flavonoids, and capsaicinoids. It is extensively used as a spice, natural colorant, and functional ingredient in processed food products.

Recent reviews also highlight that pepper by-products (peels and seeds) represent an important source of bioactive compounds for the development of functional foods within the circular economy concept (Anaya-Esparza *et al*., 2021; Ben Abdallah et al., 2025). Moreover, pepper is widely used in sauces, canned foods, and natural food additives, contributing to value-added agro-industrial applications (Anaya-Esparza et al., 2021). .

I.13.2 Pharmaceutical and Cosmetic Use

Recent scientific evidence shows that *Capsicum annuum* contains biologically active compounds such as capsaicin, flavonoids, and carotenoids, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties, making it highly relevant in pharmaceutical applications. It is commonly used in topical formulations for pain relief, treatment of rheumatic conditions, and stimulation of blood circulation. In cosmetics, pepper extracts are incorporated into skincare products for anti-aging and antioxidant protection effects (Mandal *et al*., 2022) .

Recent reviews also emphasize the use of pepper phenolic compounds in the development of functional cosmetic products with skin-protective and antioxidant properties (Alonso-Villegas et al., 2023).

I.13.3 Medical Use

Recent literature indicates that pepper contains bioactive compounds, particularly capsaicin, which exhibits multiple medicinal properties including analgesic, anti-inflammatory, anticancer, and cardioprotective effects.

Consumption of pepper has been associated with metabolic benefits, including weight management, improved metabolism, and cardiovascular health support, in addition to antioxidant and antimicrobial activities (Hong & Kim, 2020).

Furthermore, pepper is widely used in traditional medicine for the treatment of digestive disorders, pain relief, and circulatory improvement, while modern research continues to investigate its molecular mechanisms in disease prevention and therapy (Khalid & Ahmed, 2022).

I.13.4 Use as Insecticide

Recent studies demonstrate that extracts of *Capsicum annuum* possess natural insecticidal and repellent properties due to the presence of capsaicinoids and phenolic compounds.

These extracts are effective against several agricultural pests and can reduce the reliance on synthetic pesticides, making them an environmentally friendly alternative in sustainable agriculture.

Modern research highlights the potential use of pepper-derived compounds in integrated pest management (IPM) strategies and the development of botanical insecticides for crop protection (Salehi *et al*., 2018).

CHAPTER II

Aphids and the Agricultural Efficacy of ACEPLAN 20 SP

II.1 Definition of Aphids

Aphids are small insects belonging to the order Hemiptera and the family Aphididae. They are among the most important agricultural and horticultural pests worldwide. Aphids feed on plant phloem sap, causing direct damage to plants and acting as major vectors of plant viruses. In addition, their excretion of honeydew promotes the growth of sooty mold, which reduces photosynthetic efficiency and plant vigor (Blackman & Eastop, 2000).

II.2 Classification of Aphids

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Suborder: Sternorrhyncha

Superfamily: Aphidoidea

Family: Aphididae

Genus: Aphis

Species: Aphis gossypii Glover

(Brumley, 2020).

II.3 Economically Important Aphid Species and Their Host Plants:

Table II.1: Economically important aphid species and their host plants (Gebretsadik *et al*., 2025).

Aphid species	Host plants
Aphis gossypii	GoSsVpium hirsutum. *Cucumis sativus. Capsicum annuum. Solanumtuberosum, Nicotiana tabacum*
Acyrthosiphon gossypii	*Gossypium hirsutum*, and lequmes
Myzus persicae	Brassicaceae, Solanaceae, Cucurbitaceae, Rosaceae family
Diuraphis noxia	Poaceae family (*Triticum Aestivum, Hordeum vulgare*)
Rhopalosiphum maidis	Poaceae family (*Sorghum bicolor, Zea mays*)
Rhopalosiphum padi	Poaceae family (*Triticum Aestivum*)
Rhopalosiphum rufiabdominalis	*Triticum Aestivum, Sorghum bicolor, Hordeum vulgare*
Sitobion miscanthi	Poaceae family (*Triticum Aestivum*)
Aphis glycines	*Glycine max, Solanum tuberosum, brassica rapa*
Aphis fabae	*Vicia faba*
Aphis craccivora	*Medicago sativa, Robinia pseudoacacia*
Therioaphis maculata	*Medicago sativa*
Schizaphis graminum	Over 70 graminaceous species, including *Triticum Aestivum, Hordeumvulgare, Sorghum bicolor, Zea mays*
Melanaphis sacchari	*Sorahum bicolor*
Acyrthosiphon pisum	*Vicia fabae, Pisum sativum, Medicago sativa, Trifolium pratense,*

II.4 Life Cycle:

The life cycle of Acyrthosiphon pisum begins in spring when overwintered fertilized eggs hatch into wingless females known as the fundatrix, which immediately starts reproducing through parthenogenesis without the need for males. During spring and summer, the species reproduces asexually, producing genetically identical female offspring, and the nymphs pass through four instars before reaching adulthood.

Under favorable environmental conditions such as high temperatures and long day length, rapid population growth occurs due to continuous parthenogenetic reproduction. In late summer and early autumn, decreasing day length and changing environmental conditions trigger a shift to sexual reproduction, resulting in the production of sexual females and males (winged or wingless). After mating, females lay fertilized eggs that enter diapause to survive winter conditions (Reddy *et al*., 2020).

II.5 Types of Reproduction In the Pea Aphids:

II.5.1 Asexual Reproduction (Parthenogenesis)

During spring and summer, female aphids reproduce without mating and produce genetically identical daughters. The offspring pass through four nymphal instars before becoming adults. Females begin reproducing 7–15 days after birth. Adult females can produce 50–100 nymphs at a rate of 6–7 nymphs per day (Reddy *et al*., 2020).

II.5.2 Sexual Reproduction:

Sexual reproduction occurs in autumn as a response to shorter day length and hormonal changes. During this period, sexual females and winged or wingless males are produced. After mating, females lay fertilized eggs that overwinter and hatch the following spring, initiating a new life cycle (Reddy *et al*., 2020)

II.5.3 Aphid-plant interaction (the showdown of supremacy) :

The interaction between plants and aphids is a complex relationship resulting from long-term coevolution between both partners, where plant chemical and physiological defenses influence aphid adaptation and host specialization. Aphids have developed various mechanisms to overcome plant defenses, while plants, in turn, have evolved multiple defense strategies, including physical barriers, chemical compounds, and immune responses to reduce infestation. This continuous interaction is described as an evolutionary arms race, in which each side constantly develops new strategies to counter the other As shown in the Figure II.1: (Gebretsadik *et al*., 2025).

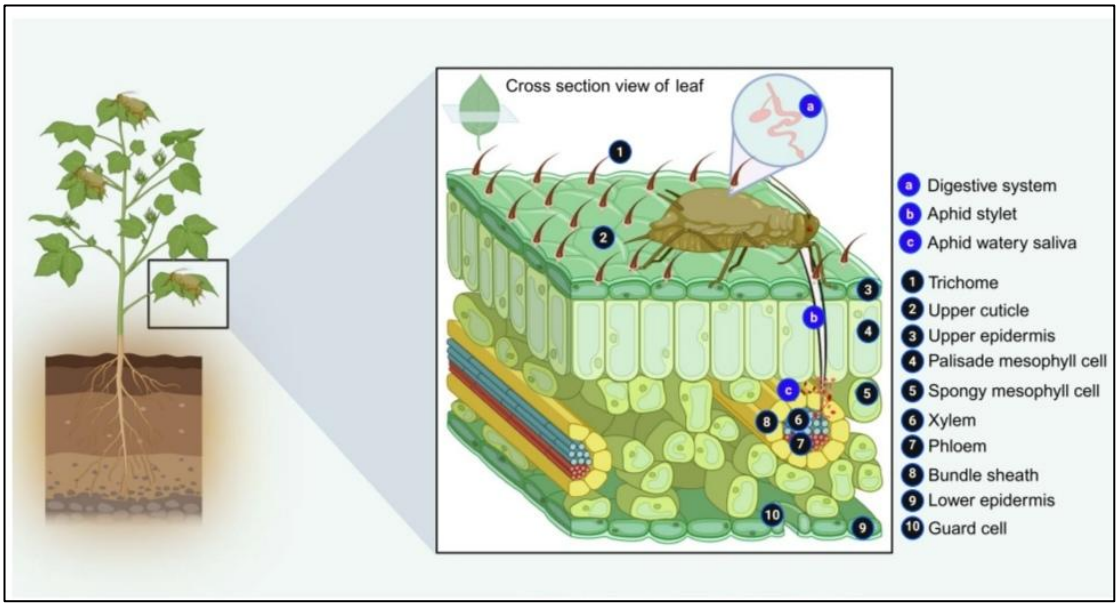

Figure II.1: Graphic illustration of the aphid-plant interaction system.

(1) Trichomes; (2) The upper cuticle, which serves as a physical barrier or chemical Deterrent to aphids; (3) The upper epidermis; (4) The palisade mesophyll cells, which contain dense cells and thicker cell walls, providing additional Physical barriers or chemical deterrents to protect plants from aphid stylet penetration; (5) The spongy mesophyll cells; (6) The xylem; (7) The Phloem; (8) The bundle sheath; (9) The lower epidermis; (10) The guard cells. (a) Digestive system; (b) Aphid stylet; (c) Watery saliva, which is secreted By aphids into sieve tubes when ingesting phloem sap. This saliva helps protect capillary food canals from clogging with phloem proteins And detoxifes defensive substances in the phloem. Aphids use their stylets to penetrate plant tissues, which are adapted for piercing cell walls And accessing the nutrient-rich phloem sap. Aphids inject saliva containing a complex mixture of proteins that suppress plant immune defenses. In Turn, plants detect these salivary secretions via molecular patterns, triggering defense signaling pathways. Upon detection of aphid presence, plants Produce toxic compounds or proteins that directly harm or inhibit aphids. Additionally, plants may develop thicker cell walls to impede aphid stylet Penetration and release volatile VOCs that attract natural enemies.

II.6 Damages Caused by Aphids:

II.6.1 Direct damage:

It feeds by sucking plant sap from leaves, stems, and pods, and attacks seedlings, terminal shoots, flowers, and pods. This results in chlorosis, leaf curling, wilting, nutrient deficiency, and plant stunting. Damage is also caused by the injection of phytotoxic saliva through its piercing-sucking mouthparts (Reddy *et al*., 2020).

II.6.2 Indirect damage:

It acts as a vector of more than 30 plant viruses, including cucumber mosaic virus, beet yellows virus, pea enation mosaic virus, and bean leafroll virus. In addition, honeydew secretion promotes the growth of sooty mold on leaves, which reduces photosynthesis efficiency (Reddy *et al*., 2020).

II.6.3 Economic damage:

Yield losses of up to 35.7% have been reported in pea crops. Significant economic losses also occur in pulses such as peas, lentils, and faba beans due to reduced yield and quality As shown in the Figures II.2.3: (Reddy *et al*., 2020).

Figure II.2: Acyrthosiphon pisum on pea leaf (left) and a close up of an aphid on alfalfa flower (right). Photos: Dr. John Gavloski

Figure II.3: Severe infestation of pea aphids causing wilting of fababean (left) and severe damage by pea aphids leaving fababeans podless by harvest (right).Photos: Dr. Tyler Wist.

II.7 Current Control Strategies and Challenges:

Current management of aphid Infestations in field or greenhouse relies on an integrated approach that includes, but is not limited to, synthetic chemical pesticides, biological agents, plant resistance, cultural practices, biorational compounds, and RNAi. While each of these strategies contributes to reducing aphid populations, significant challenges and limitations remain.

II.7.1 Synthetic Chemical Control:

Chemical pesticides are widely used for aphid control due to their fast and strong effectiveness, but their efficiency varies and their overuse has led to resistance development in aphid populations (Diaz *et al*., 2026). They may also harm beneficial organisms, although alternative application methods can reduce environmental impacts (Diaz *et al*., 2026). To manage resistance, strategies such as pesticide rotation and the development of new compounds and molecular tools like RNAi are recommended (Andualem & Asaye, 2023; Singh *et al*., 2020).

II.7.2 Biological Control:

Biological control is an important alternative to chemical pesticides for aphid management and includes the use of predators, parasitoids, and microbial pathogens such as fungi, bacteria, and viruses (Diaz *et al*., 2026). It can be implemented through three main strategies: classical control (introducing exotic natural enemies), augmentative control (mass release of reared agents), and conservation control (enhancing existing natural enemies).

These agents have shown strong effectiveness in reducing aphid populations, including lady beetles, lacewings, parasitoid wasps, and entomopathogenic fungi that can cause high mortality under favorable conditions. However, their effectiveness is not always consistent, as it is influenced by environmental conditions, mass-rearing challenges, and poor establishment in the field, which limits their long-term sustainability (Diaz *et al*., 2026).

II.7.3 Plant Resistance for Aphid Contro:

Plant resistance to aphids relies on intrinsic defense mechanisms that reduce infestation, feeding, and damage through three main types: antibiosis (reducing aphid survival and reproduction), antixenosis (deterring feeding and colonization), and tolerance (limiting damage without directly affecting aphids) (Cardona *et al*., 2022; MacWilliams *et al*., 2023).

These defenses include physical barriers such as trichomes, waxy surfaces, and strong cell walls, as well as chemical defenses based on secondary metabolites like acylsugars and

benzoxazinoids that inhibit aphid performance and attraction. In addition, genetic defenses such as resistance (R) genes andimmune receptors activate plant immune responses against aphid attack (Cardona *et al*., 2022; MacWilliams *et al*., 2023).

Overall, plant resistance provides a sustainable and effective strategy for aphid management by integrating physical, chemical, and genetic defense mechanisms

II.7.4 Cultural Control :

Cultural control is a key component of integrated pest management and relies on agricultural practices such as irrigation and fertilization management, crop rotation, planting time adjustment, and crop diversification to reduce aphid populations (Sandhi & Reddy, 2020).].

Important strategies include intercropping, cover cropping, companion planting, and mulching, which help disrupt aphid establishment and enhance natural enemies, leading to reduced infestations (Lamzira *et al*., 2025; Saldanha *et al*., 2025).

Although effective, these practices depend on a good understanding of field ecosystem interactions and may be limited by pest interactions or unstable biological control agents. Therefore, they are often combined with other contromethods to improve overall effectiveness (Mollaei *et al*., 2020; Kheirodin *et al*., 2024)

II.7.5 Biorational Control:

Biorational control is an eco-friendly aphid management approach that relies on semiochemicals (behavior-modifying signals like plant volatiles) and biopesticides (natural compounds from plants, microbes, or minerals) to repel aphids or reduce their survival and reproduction (Diaz *et al*., 2026).

Plant volatiles—whether constitutive or induced—can directly deter aphids, attract their natural enemies, or even act as toxic agents at high concentrations, while herbivore- or virus-induced volatiles can also alter aphid behavior and plant defenses responses (Wang *et al*., 2021; Yi *et al*., 2023; Casas *et al*., 2025; Hu *et al*., 2022).

However, aphids can overcome these defenses through detoxification, behavioral adaptation, or manipulation of plant signaling, making long-term control complex. Therefore, effective strategies must consider the evolutionary interactions between plants, aphids, and pathogens to improve sustainability (Diaz *et al*., 2026).

II.7.6 RNAi for Aphid Control:

RNA interference (RNAi) is a gene-silencing technology that uses doublestranded RNA to block essential aphid genes, leading to reduced growth, reproduction, and often mortality (Diaz *et al*., 2026).

It has shown strong p-otential in aphid management by targeting metabolic, developmental, and salivary genes, using different delivery methods such as spraying, feeding, and transgenic plants (Diaz *et al*., 2026).

However, its effectiveness varies depending on aphid genotype and delivery method, and concerns remain about off-target effects and environmental safety, requiring improved design and higher precision for reliable field application

II.7.7 Targeting Symbiosis for Aphid Control:

Aphids depend on symbiotic bacteria for nutrition, stress tolerance, and defense, so disrupting these symbioses can strongly weaken their survival and performance Targeting symbiont-related genes or pathways can reduce nutrient exchange and even cause aphid mortality.

Some symbionts also provide protection against heat stress and natural enemies, so their disruption can increase the effectiveness of biological control agents (Kucuk *et al*., 2025; Ali *et al*., 2022).

In addition, certain symbionts can be engineered as delivery systems for RNAi-based control, although their manipulation is still limited by technical challenges in culture and genetic modification (Dyson *et al*., 2022; Feng & Wilson, 2024).

III. Agricultural Efficacy of ACEPLAN 20 SP

III.1 Definition

Acetamiprid 20% SP is a soluble powder formulation containing 20% of the active ingredient Acetamiprid. Acetamiprid 20% SP is a highly effective systemic insecticide for the control of aphids, jasssids and whiteflies.

Table III.1. Technical Data Sheet for Drug Identification

NOMBRE COMUN	**Acetamiprid 20% SP**
INGREDIENTE ACTIVO	Acetamiprid
GRUPO QUIMICO	Neonicotinoides
FORMULACION	Polvo Soluble (SP)
COMPOSICION	Acetamiprid 20% p/p (200 g/kg) Coformulantes c.s.p. 100% p/p (1 kg)
MODO DE ACCION	Sistémico, ingestión y contacto.
TOXICIDAD	Grupo II, moderadamente peligroso.

❖ **There are many pharmaceutical forms, including:**

III .2 Chemical Composition

Table III.2: represents the chemical composition of the drug

Acetamiprid a.i.	20.00 % w/w
Blend of Sodium Dodecyl Benzene Sulfonate	4.50 %
Sodium Sulphate & Sodium alkylate Mixture	% w/w
Sodium Citrate, Dihydrate	30.00 % w/w
Calcium Hydrogen Phosphate Dihydrate	0.10 % w/w
Colouring agent (Brilliant Blue)	0.05 % w/w
Lactose Monohydrate	Q.S %

III.3 Mechanism of Action:

Acetamiprid acts through a precise neurotoxic mechanism by binding to nicotinic acetylcholine receptors (nAChRs) in the insect nervous system, causing continuous nerve stimulation and overstimulation, which disrupts normal neural transmission. This results in progressive paralysis followed by insect death. It is a systemic insecticide that is absorbed by plant tissues and translocated through the sap, providing long-lasting protection to both treated and untreated parts of the plant. In addition, it is effective through both contact and ingestion during pest feeding on plant tissues.

This mode of action is well documented for neonicotinoid insecticides, which target insect nAChRs and induce persistent neural activation leading to neurotoxicity and mortality (Ihara *et al.*, 2017; Taillebois *et al.*, 2018). Studies also confirm the systemic behavior of acetamiprid within plants and its effectiveness against sap-feeding insects (Zuščíková *et al.*, 2023; Wallace, 2014).

III .4 Uses (Field Applications)

Scientific studies indicate that ACEPLAN 20 SP (active ingredient: acetamiprid 20%) is widely used in modern agricultural systems due to its high efficacy and systemic properties. It is applied on a broad range of economically important crops, including tomato, potato, cotton, fruit trees (apple and citrus), as well as various vegetable crops, in order to protect agricultural production from insect pest damage. Scientific references confirm that this compound is commonly used in vegetable, fruit, and field crop protection because of its ability to be absorbed and translocated within plant tissues, providing long-lasting systemic protection (Jeschke & Nauen, 2008; IRAC, 2023; FAO Plant Protection Guidelines).

In addition, acetamiprid is primarily targeted against sap-feeding insect pests, which are among the most economically destructive agricultural pests, including aphids, whiteflies, thrips, and leafhoppers. It acts on the insect nervous system by binding to nicotinic acetylcholine receptors, leading to overstimulation, paralysis, and ultimately death (Horowitz *et al*., 2020; Tomizawa & Casida, 2005). Therefore, acetamiprid is considered an important component of Integrated Pest Management (IPM) programs for improving crop protection and agricultural productivity (Simon-Delso *et al*., 2015).

III .5 Application Method

Application methods of acetamiprid play a crucial role in determining the efficiency of pest control and minimizing environmental contamination. Acetamiprid is generally applied through foliar spraying because of its systemic activity and its effectiveness against sucking insect pests such as aphids, whiteflies, and leafhoppers. Proper preparation of the spray solution, including accurate dilution and homogeneous mixing, is essential to ensure uniform distribution of the insecticide on plant surfaces. Recent studies have demonstrated that the addition of suitable adjuvants improves the physicochemical properties of acetamiprid spray solutions and enhances droplet deposition efficiency (Zeeshan *et al*., 2024). Moreover, modern spraying technologies, including unmanned aerial vehicle (UAV) systems, have been increasingly adopted to optimize pesticide application and reduce spray drift. Environmental conditions such as temperature, humidity, and wind speed are also important factors influencing the effectiveness of treatment and pesticide persistence on plant surfaces (Liu *et al*., 2024). During application, safety precautions including the use of personal protective equipment are necessary to reduce risks to operators and non-target organisms (Zuščíková *et al*., 2023).

III .6 Toxicity and Biological Activity

Toxicity and biological activity are among the most important characteristics determining the effectiveness and environmental safety of insecticides such as acetamiprid. Acetamiprid exhibits strong insecticidal activity against a wide range of agricultural pests, particularly sucking insects, through its action on nicotinic acetylcholine receptors (nAChRs) in the insect nervous system, resulting in paralysis and death of target insects. Its systemic and translaminar properties contribute to prolonged biological efficacy and efficient pest control even at relatively low concentrations (Li *et al*., 2024).

Recent studies have also highlighted that the biological activity of acetamiprid is influenced by several factors, including concentration, formulation type, environmental conditions, and susceptibility of the target organisms. Despite its effectiveness against pests,

concerns have been raised regarding its toxicity toward non-target organisms such as pollinators, beneficial insects, aquatic species, and soil microorganisms. Chronic or excessive exposure may lead to ecological disturbances, oxidative stress, behavioral changes, and impairment of reproductive and immune functions in non-target species (Zuščíková *et al*., 2023; EFSA, 2024).

Furthermore, environmental persistence and residue accumulation of acetamiprid in soil and water ecosystems may increase the risk of contamination and contribute to the development of insect resistance. Therefore, proper management strategies and controlled application methods are necessary to optimize insecticidal efficiency while minimizing environmental and toxicological risks (Liu *et al*., 2024).

III .7 Environmental Impact

ACEPLAN 20 SP is a systemic insecticide formulated on the active ingredient acetamiprid, a compound belonging to the neonicotinoid class of insecticides. According to recent regulatory and scientific assessments, including updated evaluations by the European Food Safety Authority (EFSA, 2021) and the U.S. Environmental Protection Agency (EPA, 2022), acetamiprid remains widely used in agricultural systems due to its effectiveness against sap-feeding insect pests; however, its environmental profile continues to raise significant ecological concerns.

From an environmental fate perspective, acetamiprid demonstrates moderate water solubility and mobility in soil, which increases its potential for transport through soil profiles and eventual contamination of surface and groundwater systems. Recent regulatory reviews by EFSA (2021) and ECHA (2023) indicate that under conditions of intensive or improper application, the compound may persist long enough in environmental compartments to pose risks to aquatic ecosystems, particularly through runoff and leaching processes.

Ecotoxicological evidence summarized in recent reviews, including Kumar *et al*. (2022) and Wood & Goulson (2023), highlights that neonicotinoids such as acetamiprid can negatively affect non-target organisms. Pollinators, especially Apis mellifera, are highly sensitive, with sublethal exposure associated with impaired foraging behavior, reduced learning capacity, and altered navigation. These effects may contribute to colony-level stress and indirectly impact biodiversity through disruption of plant–pollinator interactions. In addition, soil organisms and microbial communities may also be affected, leading to disturbances in nutrient cycling, organic matter decomposition, and overall soil ecosystem functioning.

Furthermore, repeated and extensive use of ACEPLAN 20 SP may accelerate the development of resistant pest populations, as highlighted in recent FAO guidance (FAO, 2022). This resistance phenomenon can reduce long-term field efficacy and lead to increased pesticide applications or substitution with alternative compounds that may carry higher environmental risks. Consequently, modern regulatory frameworks strongly emphasize mitigation strategies, including strict adherence to application rates, establishment of buffer zones near aquatic environments, and the implementation of Integrated Pest Management (IPM) approaches to reduce reliance on chemical control and minimize ecological impacts.

III .8 Storage and Safety

Proper storage of acetamiprid (ACEPLAN 20 SP) is essential to maintain its chemical stability and biological efficacy. The product should be kept in a cool, dry, and well-ventilated place, away from direct sunlight, moisture, and heat sources, and always stored in its original tightly sealed container to prevent degradation and contamination. Studies indicate that improper storage conditions, particularly high temperature and humidity, can accelerate the breakdown of the active ingredient and significantly reduce its insecticidal performance (FAO, 2023).

The shelf life of acetamiprid formulations depends strongly on storage conditions and formulation type. Under recommended conditions, the product may remain stable and effective for several years; however, exposure to extreme environmental conditions can reduce its chemical stability and biological activity over time. Such conditions may also promote the formation of degradation products that could affect both safety and performance (FAO, 2023; Zhang *et al*., 2024).

During handling and application, strict safety measures must be followed to minimize human exposure. Users are required to wear personal protective equipment (PPE), including gloves, masks, protective clothing, and eye protection, while avoiding direct contact, inhalation, and ingestion. Although acetamiprid is generally considered less toxic than other neonicotinoids, improper handling can still pose acute and chronic health risks, highlighting the importance of adherence to established safety guidelines (EFSA, 2024; Zuščíková *et al*., 2023).

Finally, safe disposal of containers and residues is crucial to prevent environmental contamination. Empty containers should be triple-rinsed and disposed of according to local regulations or pesticide waste management programs, while leftover spray solutions must never

be discharged into soil or water bodies. Improper disposal can contribute to environmental pollution and negatively affect non-target organisms, including aquatic species and beneficial insects (Liu *et al*., 2024).

SECOND PART

Experimental Section

CHAPTER I

Materials and Methods

I.1 Materials

I.1.1 Chemical Materials

In the applied part of this study, we used numerous chemical products and reagents, as well as various laboratory equipment and instruments, as shown in Table (01).

Table I.1: Equipment, culture media, chemical products, and reagents

ChemicalProducts and Reagents	Materials and equipment
▪ Acetamiprid(Aceplan 20 SP) ▪ Ascorbic acid ▪ Trichloroacetic acid (10%) ($C_2HCI_3O_2$) ▪ Baird-Parker agar ▪ Sodium carbonate (Na2CO3)(%7.5) ▪ Aluminum chloride ($AICI_3$)(%2) ▪ Ferric chloride ($FeCl3 \cdot 6H_2O$) (pure, 0.1%) ▪ DPPH ▪ Diclofenac ▪ Distilled water ▪ Ethanol ▪ Potassium ferricyanide (K3Fe(CN))(%10) ▪ Folin-Ciocalteu reagent(%10) ▪ Hydrochloric acid (HCl, concentrated) ▪ Methanol ▪ Phosphate-buffered saline (PBS), pH = 6.4 ▪ Phosphate buffer (0.2 mol/L, pH = 6.6) ▪ Vanillin	▪ Magnetic stirrer ▪ Water bath ▪ Electronic balance ▪ Magnetic bar ▪ Beaker ▪ Scalpel ▪ Petri dish ▪ Electric grinder ▪ Centrifuge ▪ Cotton ▪ Crystallizing dish ▪ Erlenmeyer flask ▪ Funnel ▪ Filtration funnel ▪ Glass vial ▪ Micropipettes ▪ Microcentrifuge tubes ▪ Aluminum foil ▪ Filterpaper ▪ Hot plate ▪ Refrigerator ▪ Spatulas ▪ Spectrophotometer ▪ Sprayers ▪ Test tubes ▪ Watch glass

I.1.2 Biological material

I.1.2.1 Plant material studied

The species *Capsicum annuum* was collected in November 2025 in the region of Al-Rabbah, El Oued, and identified by Professor Chouikh Atef, a botanist at the University of El Oued As shown in the (Figure I.1).

Figure I.1: The chili pepper plant (*Capsicum annuum L.*)

Three distinct specimens were selected based on their stage of development, then dried for two months in conditions protected from direct sunlight and moisture.

The fruits were subsequently ground into powder using an electric blender and stored in airtight containers until further use As shown in the (Figure I.2)

unripe pepper **ripe pepper** **the intermediate maturity stage**

Figure I.2: Powder derived from unripe, intermediate maturity stage, and ripe peppers.

I.1.2.2 Aphid Insects

Aphids are among the most important pests of both wild and cultivated plant species, and their populations are expected to increase as a result of climate change. Rising temperatures accelerate their reproduction and enhance their fecundity. Aphids are specialized insects that

feed on the phloem sap of vascular plants. They therefore have a direct impact on the vigor of the host plant, mainly due to the loss of sugars and defense compounds (secondary metabolites) that are abundant in the sap. Aphids are also vectors of diseases: owing to their feeding mode (sap-sucking herbivores), they can transmit pathogens into the tissues of the host plant (Claros Cuadrado *et al*., 2019). The aphids used in the present study were collected from a farm located in the Nakhla municipality, El Oued province (Algeria). Taxonomic identification of the collected specimens was carried out by Dr. Babaousmail mahfoud based on their morphological characteristics As shown in the Figure I.3:

Figure I.3: An image of an aphid

I.2 Methods

The present study was conducted on extracts obtained through selective and specific extraction procedures aimed at isolating distinct families of secondary metabolites. Extracts enriched in polyphenols, flavonoids, and tannins were prepared and analyzed.

Thereafter, the biological activities of the obtained extracts were investigated through a series of bioassays, including antioxidant activity, *in vitro* anti-inflammatory activity evaluated by the egg albumin denaturation method, as well as toxicity assessment.

The different experimental analyses performed in this study aimed to assess the biological effectiveness of the prepared extracts and to explore their possible applications in pharmaceutical and biological fields.

I.2.1 Extraction

I.2.1.1 Preparation of extracts by maceration

Assisted maceration extraction (MAE) was carried out according to published methods (Tlili and Benine, 2022).

Ten grams of peppers of Different age groups (ripe pepper ;Inrip pepper; intermediate maturity stage) were used with 100 mL of distilled water in a beaker at room temperature for 24 hours. The beakers were completely covered with aluminum foil to prevent the degradation of photosensitive compounds.

After filtration using filter paper, the extract was dried at room temperature for at least 72 hours, at a temperature not exceeding 45 °C. Finally, the dry residue was collected in amber tubes and stored at room temperature As shown in the Figures I.4.5:

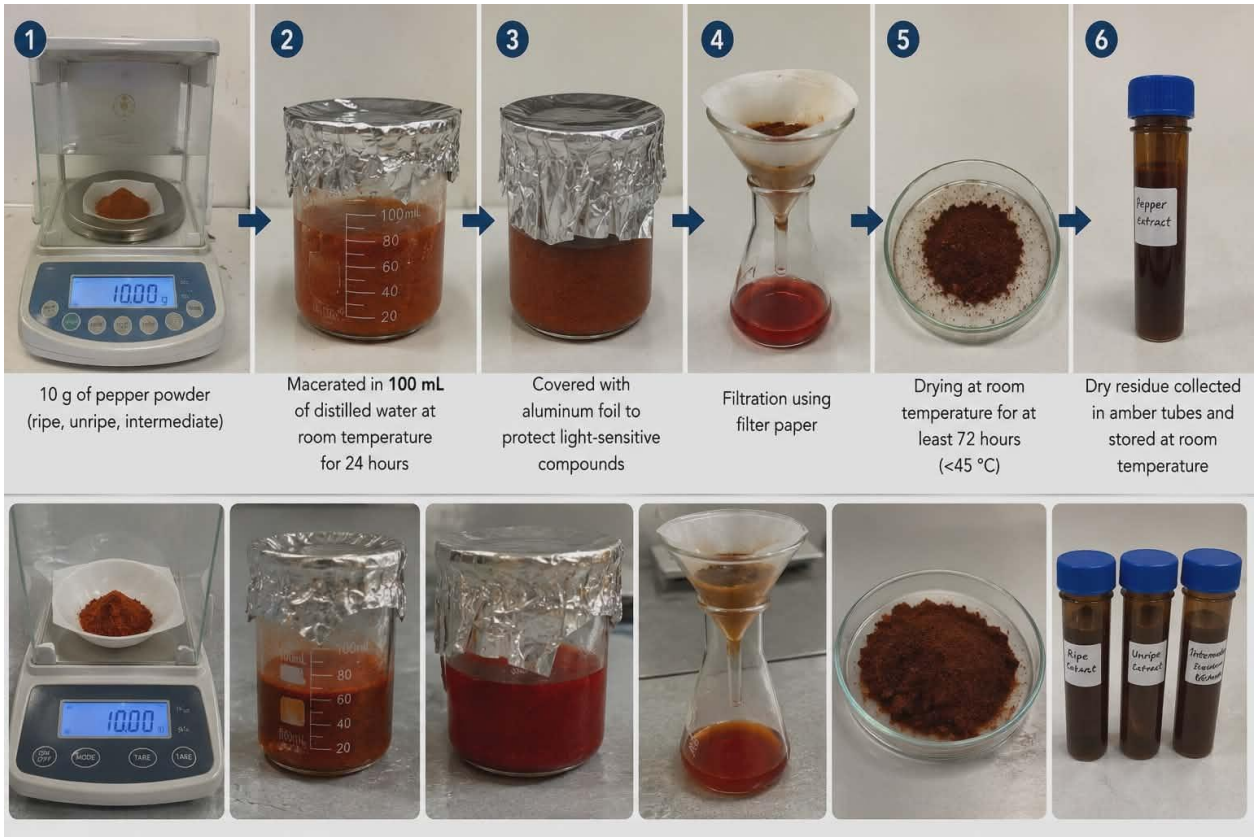

Figure I.4: Diagram illustrating the extraction process of plant parts.

Figure I.5: extract of different parts of *Capsicum annuum* plant.

I.2.2 Determination of phenolic compounds

Phenolic compounds are a diverse group of secondary metabolites widely distributed in the plant kingdom, characterized by the presence of one or more hydroxyl groups directly attached to an aromatic ring (Smith *et al*., 2023). These compounds are of considerable interest due to their strong antioxidant potential and their reported biological activities, including antimicrobial, anti-inflammatory, and anticancer properties (Zhang *et al*., 2024).

I. 2.2.1Determination of total polyphenols

7The determination of total phenolic content is commonly based on the colorimetric Folin–Ciocalteu method, which relies on the reduction of the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, where these compounds act as reducing agents (Singleton *et al*., 1999; George *et al*., 2022).

This reaction leads to the formation of a blue-colored complex, which can be quantified spectrophotometrically at a wavelength ranging from 760 to 765 nm (Li *et al*., 2023).

The intensity of the developed color is directly proportional to the total phenolic concentration in the sample, and the results are typically expressed as gallic acid equivalents (GAE) using a standard calibration curve (Zhang *et al*., 2024).

The total phenolic content was determined using the Folin–Ciocalteu method as described by Singleton and Rossi (1965), with slight modifications based on Chaves *et al*. (2020). For each extract, 0.2 mL of sample was mixed with 1 mL of Folin–Ciocalteu reagent (10%). Then, 0.8 mL of Na_2CO_3 solution (7.5%) was added to the mixture.

The mixtures were incubated at room temperature in the dark for 30 min.

The absorbance was measured at 760 nm using a UV–Vis spectrophotometer.

A calibration curve was prepared using gallic acid solutions at concentrations ranging from 0.005 to 0.02 mg/mL.

I. 2.2.2**Determination of condensed tannins**

The determination of condensed tannins is commonly performed using spectrophotometric colorimetric assays, particularly the vanillin–hydrochloric acid (vanillin–HCl) and butanol–hydrochloric acid (butanol–HCl) methods. These assays are based on the acid-catalyzed transformation of proanthocyanidins into colored derivatives, mainly anthocyanidin-type compounds, which can be quantitatively measured using UV–visible spectrophotometry (Amarowicz & Pegg, 2024).

In the vanillin–HCl assay, condensed tannins react with vanillin in a strongly acidic medium to form red-colored complexes, the intensity of which is measured at approximately 500 nm. The color development is directly proportional to the concentration of condensed tannins present in the sample and is typically quantified using calibration curves prepared with catechin or procyanidin standards, with results expressed as catechin equivalents (Liang *et al*., 2024).

According to the method described by Kebsa *et al*., (2024), with slight modifications, 0.1 mL of each sample or standard is added to 0.75 mL of a vanillin solution (4% in methanol) and 0.375 mL of concentrated hydrochloric acid (HCl). The mixture is incubated for 15 minutes, and the absorbance is measured at 500 nm. The concentrations of condensed tannins are determined from calibration curves established using catechin (0–0.5 mg/mL) as a reference.

1.2.2.3**Determination of flavonoids:**

The determination of total flavonoid content (TFC) is most commonly performed using spectrophotometric colorimetric assays based on complex formation between flavonoid molecules and metal ions, particularly aluminum chloride ($AlCl_3$). This method relies on the ability of flavonoids containing hydroxyl and keto functional groups to form stable acid–metal complexes with Al^{3+} ions, resulting in the development of a yellow chromophore that can be quantified using UV–visible spectrophotometry (Gao *et al*., 2025).

The intensity of the formed complex is directly proportional to the flavonoid concentration in the sample and is typically measured at wavelengths ranging from 415 to 430 nm. Quantification is achieved through calibration curves prepared using standard compounds such as quercetin or rutin, and results are expressed as quercetin equivalents (QE) (Erol & Irmisch, 2025).

The total flavonoid content was determined using a colorimetric method based on aluminum chloride according to Ordonez *et al*. (2006), and in agreement with recent studies reporting its application in phytochemical and antioxidant evaluations (Thepphakhun&Intanon, 2020), with slight modifications.

In this method, 0.75 mL was mixed with 0.75 mL of aluminum chloride ($AlCl_3$) solution (2% in methanol) in test tubes. After incubation for 1 hour at room temperature in the dark, the absorbance was measured at 415 nm using a UV–Vis spectrophotometer.

Quercetin (0.01–0.04 mg/mL) was used as a reference standard

I.2.3 Evaluation of biological activities

I.2.3.1 Antioxidant activity

The antioxidant activity was evaluated using two different methods: free radical scavenging (DPPH) assays and ferric reducing antioxidant power (FRAP).

I.2.3.1.1 DPPH assay

The antioxidant activity of chili pepper plant extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by Baliyan *et al*. (2022) with slight modifications.

A stock solution of DPPH was prepared by dissolving 4 mg of DPPH in 50 mL of ethanol. The solution was then filtered to obtain a clear working solution, which exhibited an absorbance of approximately 0.819 at 517 nm.

For the assay, 0.75 mL of the DPPH working solution was mixed with 0.75 mL of the plant extract at different concentrations ranging from 1 to 10 mg/mL. A control solution was prepared by mixing 0.75 mL of DPPH solution with 0.75 mL of ethanol. The reaction mixtures were incubated in complete darkness for 30 minutes at room temperature to prevent light-induced degradation.

After incubation, the absorbance was measured at 517 nm using a UV–Vis spectrophotometer. All measurements were carried out under identical conditions to ensure accuracy. The experiment was performed in triplicate to ensure reproducibility and reliability of the results.

The percentage of DPPH radical scavenging activity (RSA) or antioxidant activity was calculated using the following equation :

$$\%\ \text{Antioxidant activity} = [(\text{Ac} - \text{As}) / \text{Ac}] \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the sample.

Ascorbic acid was used as a standard antioxidant for comparison, with concentrations ranging from 0 to 0.07 mg/Ml.

This assay is widely used to evaluate free radical scavenging capacity, which plays a crucial role in preventing oxidative stress-related damage in biological systems (Valko *et al*., 2007).

I.2.3.1.2 FRAP assay

The antioxidant capacity, namely the ferric reducing power (Fe^{3+}), was evaluated in the extracts according to the method described by Meir S. Oyaizu (1986), with slight modifications in accordance İlhami Gülçin and Saleh H. Alwasel (2025).

For each extract, different concentrations ranging from 1 to 10 mg/mL were prepared. Then, 0.5 mL of each sample solution was mixed with 0.6 mL of phosphate buffer solution (0.2 M, pH = 6.6) and 0.6 mL of potassium ferricyanide [$K_3Fe(CN)_6$] solution (1%, w/v).

The reaction mixtures were incubated in a water bath at 50 °C for 20 minutes. Subsequently, 0.5 mL of(10%) trichloroacetic acid (TCA) was added to stop the reaction, and the mixture was centrifuged at 3000 rpm for 10 minutes.

After centrifugation, 0.6 mL of the supernatant was collected and mixed with 0.6 mL of distilled water and 0.6 mL of an aqueous ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution (0.1%, w/v).

The absorbance of the final solution was measured at 700 nm using a UV–Vis spectrophotometer against a blank prepared under the same conditions.

The test was repeated three times to ensure accuracy and provide reliable results.

I.2.3.2 Insecticide efficacy assay

Sampling of aphids and field experiments were conducted in El Oued province (R'bah district, Nakhla municipality) in February 2026. A group of 600 aphids was randomly collected from more than 10 adult plants of *Lemon Verbena*. Immediately after collection, the insects were carefully transferred in plastic containers to the laboratory (Laboratory of Experimental Trials at the Faculty of Biology, El Oued university) for toxicity tests on the same day of collection. For this purpose, diluted extracts for all age groups and the insecticide acetamiprid, branded as ACEPLAN 20 SP, were prepared using distilled water, as well as a control sample treated only with distilled water. This resulted in four different concentrations for each compound. Specifically, the concentrations (v/v) were 1%, 5%, 10%, and 20% for extracts, and 0.075%, 0.05%, 0.025%, and 0.1% for acetamiprid. To test the insecticides in this study, 10 adult aphids were exposed to each dilution of these biopesticides in Petri dishes (10 cm diameter and 15 mm depth). Each treatment was replicated three times. The biopesticides were applied only once, by spraying equivalent dilutions of 1 mL per Petri dish, then the number of dead

insects in each Petri dish was recorded after 30 minutes and after 2 hours of spraying As shown in the Figure I.6: (Cuadrado *et al* 2019).

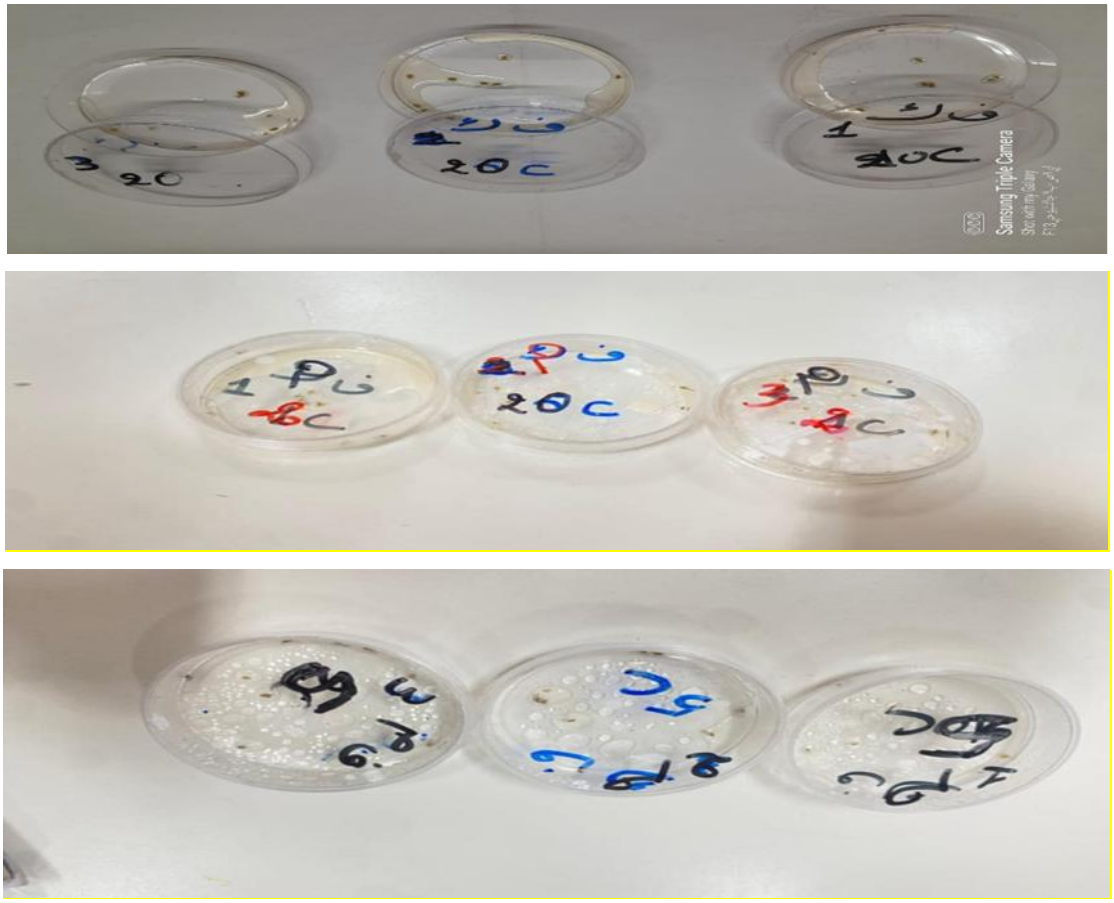

Figure I.6: Laboratory treatment of different age groups with capsaicin extracted from *Capsicum annuum*.

I.2.3.3 Anti-inflammatory activity (Albumin assay test)

The anti-inflammatory activity can be evaluated by the inhibition of protein denaturation, a mechanism first described by **Ahmed *et al*., (2020).** slight modifications. A volume of 1 mL of plant extract is mixed with 0.75 mL of a 1% albumin solution and 0.75 mL of phosphate buffer saline (PBS) at pH 6.4. The mixture is then incubated at 37 °C for 15 minutes, followed by heating at 70 °C for 5 minutes. After cooling to room temperature, the absorbance is measured at 660 nm and compared with that of the control. The percentage of inhibition is calculated to evaluate the anti-inflammatory activity of the extract, using sodium diclofenac as the reference standard.

CHAPTER II

Results and Discussion

II.1 Results and Discussion

II.1.1 Determination of phenolic compounds

Phenolic compounds are plant-derived antioxidants crucial for human health and food preservation. Their bioactive potential including anti-inflammatory, antimicrobial, and anti-carcinogenic properties makes them a vital focus in nutritional, pharmaceutical, and agricultural research. This Investigation critically evaluates the methodologies for their extraction, detection, and quantification to accurately assess antioxidant activity; In this study, a comparative analysis is was conducted among three distinct age classes defined according to clear and well-differentiated age group classifications. of hot pepper (*Capsicum annuum L.*) plants

II.1.1.1 Determination of Total Polyphenols

The total polyphenol content was determined by the spectrophotometric method adapted from Chouikh and Rebiai, (2020) using the Folin-Ciocalteureagent. The obtained results are expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g). The contents were calculated using the linear regression equation of the gallic acid calibration curve (Figure 01)

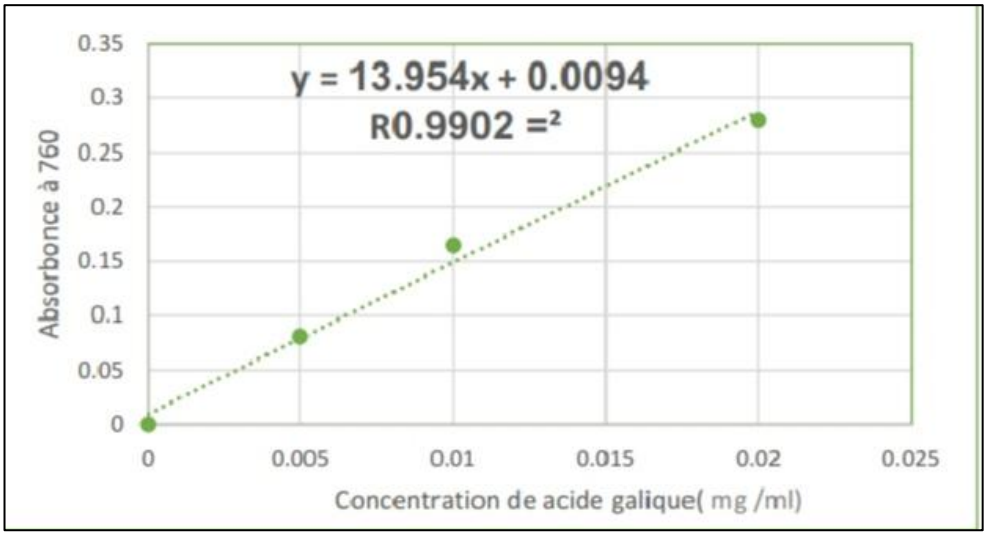

Figure II.1: Calibration Curve of Gallic Acid for the Determination of Total Polyphenols.

The results of the total polyphenol content revealed slight variations among the studied sample sizes. Both ripe and unripe samples exhibited nearly identical values, estimated at 2.23 ± 0.04 and 2.23 ± 0.04 mg GAE/g, respectively, whereas the intermediate maturity stage samples showed a slightly lower value of 2.20 ± 0.01 mg GAE/g. These findings indicate that sample size had no significant influence on the accumulation of total phenolic compounds, suggesting a relative stability of polyphenol content across the different sample sizes.

However, these results are lower than those reported by Olatunji and Afolayan (2019), who found phenolic contents ranging from 200.70 ± 11.53 to 272.47 ± 7.38 mg GAE/g in ethanolic extracts *Capsicum annuum*

Conversely, our results are higher than those reported in other studies. For instance, Yalçın *et al*. (2021) reported significantly lower phenolic contents in the pulps of certain pepper varieties, ranging from 0.075 to 0.124 mg GAE/g

II.1.1.2 Determination of flavonoids

The flavonoid content was determined by the colorimetric method described (Chouikh *et al*., 2018). From the calibration curve, we calculated the flavonoid contents of the different plant fractions, expressed in mg quercetin equivalent per gram of extract.

The flavonoid values in our study ranged from 0.51 ± 0.006 mg QE/g (the intermediate maturity stage size) to 0.69 ± 0.02 mg QE/g (ripe size), suggesting that larger fruits may accumulate slightly higher concentrations of flavonoids. These findings are consistent with those reported by Abd Rahman *et al*. (2024) regarding flavonoid levels in *C annuum* pericarp, although their reported values were notably higher (21.65 ± 1.13 mg QE/g)

For instance, Mokhtar *et al*. (2015) reported a significantly lower value of 0.098 ± 0.0015 mg QE/g for a pepper extract .

Similarly, Gámez-Mesa *et al*. (2018) demonstrated that the total flavonoid content in various pepper varieties ranged from 25.38 ± 3.44 to 60.36 ± 9.94 mg QE/100g fresh weight, which translates to approximately 0.25 to 0.60 mg QE/g .Kebu *et al*. (2024) also noted that earlier studies on *C. annuum* fruits reported flavonoid content values ranging from 0.204 to 0.962 mg QE/g

II.1.1.3 Determination of condensed tannins

Tannin content was determined according to the method of Price *et al*. (1978) and the vanillin method described by Kebsa *et al*. (2024). Vanillin reacts with free flavan-3-ol units and the terminal units of proanthocyanidins, resulting in the formation of a red-colored complex whose intensity is directly proportional to the concentration of phenolic compounds present in the intermediate maturity stage. The complex exhibits a maximum absorption at 500 nm. The obtained results show that the tannin content of pepper extract varies according to the ripening stage. Based on the calibration curve, tannin content was calculated at different ripening stages and expressed as milligrams of catechin equivalents per gram of extract (mg EC/g), as shown in Figure 8

The results indicate that the pepper extract showed a tannin content of 1.53 ± 0.23 mg EC/g, which represents the highest value compared to the other values recorded in the study.

this values are higher than those reported by Niamké *et al*. (2021), where tannin levels ranged between 0.17 and 0.25 mg/g.

On the other hand, the study by Ekom *et al*. (2021) confirmed the presence of tannins as bioactive compounds in pepper, thereby supporting the findings of the present work.

Furthermore, Choi *et al*. (2022) demonstrated that phenolic compounds vary according to the stage of ripening, which is consistent with our results showing the highest tannin content at the ripe stage and a decrease at the intermediate stage. In addition, Kim *et al*. (2021) reported that environmental and genetic factors significantly influence the content of these compounds, which may explain the discrepancies observed between different studies.

Phenolic compounds, including polyphenols, flavonoids, and tannins, are among the most important secondary metabolites that contribute to the biological activities of plants. Numerous studies have demonstrated that these compounds possess various properties, including antioxidant, antitumor, and antimicrobial activities, as well as a role in preventing diseases associated with oxidative stress (Mueed *et al*., 2023).

Moreover, the content of these compounds in plants is not constant, but is influenced by several factors such as environmental conditions, geographical location, extraction methods, and the stage of plant growth, which directly affects their biological activity (Hayat *et al*., 2020).

From a mechanistic perspective, the observed variations in total flavonoid content during the ripening stages of pepper fruits (*Capsicum annuum L*.) can be attributed to the physiological and biochemical changes accompanying fruit development. Flavonoids are secondary metabolites closely associated with plant defense mechanisms, and their accumulation varies depending on the developmental stage, which explains the differences observed amongripening stages. (Alonso-Villegas *et al*., 2023).

This variation can also be explained by the fact that the ripening process leads to the reprogramming of metabolic pathways with in the fruit, where certain phenolic compounds are either metabolized or redirected during the transition toward full ripening, or alternatively diverted to ward the biosynthesis of other compounds such as carotenoids, which in turn affects total flavonoid concentration. (Gao *et al*., 2025).

Furthermore, the differences reported in flavonoid content may be attributed to several factors, including genotype, extraction and quantification methods, as well as environmental and climatic conditions such as light intensity, temperature, soil type, and harvesting stage. Recent studies have demonstrated that these factors significantly influence the accumulation of phenolic compounds in pepper fruits (Ribes-Moya *et al*., 2020).

In addition, it has been reported that environmental stress and agronomic conditions can either stimulate or inhibit the production of phenolic compounds, including flavonoids, as part of plant defense responses against unfavorable conditions (Ninfali *et al*., 2021). Moreover, recent reports indicate that biochemical transformations during ripening lead to changes in flavonoid stability, which may explain the decrease or fluctuation in their content across developmental stages (Antioxidants Journal, 2022).

II.2 Antioxidant activity

II.2.1 DPPH assay

The DPPH assay is a widely recognized, simple, and cost-effective method for evaluating antioxidant activity. It is based on the principle that antioxidant spresent in the sample donate electrons to the DPPH free radical (1,1-diphenyl-2-picrylhydrazyl), leading to its stabilization and a noticeable color change from deep violet to pale yellow. This change is quantitatively measured using a spectrophotometer, where the degree of discoloration is directly proportional to the sample's free radical scavenging capacity, making this assay a reliable method for assessing antioxidant potential (Sadiq *et al*., 2025)

The antioxidant activity of extracts from unripe pepper, pepper at the intermediate maturity stage, and ripe pepper was evaluated using the method illustrated in Figure (02)

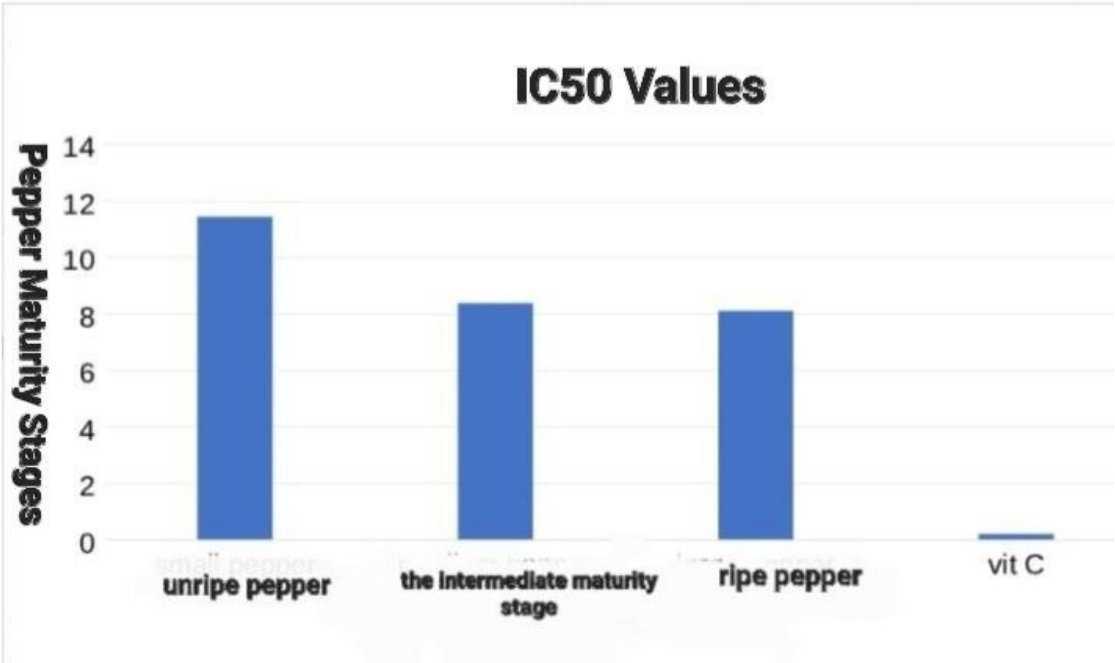

Figure II.2: Results of IC_{50} values for the DPPH radical scavengingassay.

The results show that the unripe pepper extract exhibited the highest IC50 value (11.44 ± 0.59), indicating the weakest antioxidant activity compared to the other tested samples. This was followed by the intermediate maturity stage extract with an IC50 value of (8.38 ± 0.57),

while the ripe pepper extract showed a slightly lower IC50 value (8.11 ± 0.10), suggesting a gradual improvement in free radical scavenging ability with increasing ripening stage.

In contrast, vitamin C demonstrated the strongest antioxidant activity, with the lowest IC50 value (0.21 ± 0.04), reflecting a markedly higher radical scavenging capacity compared to the plant extracts investigated in this study.

In a study in Sadiq *et al*., 2025 ,vitamin C exhibited a Reduced IC50 value compared to that obtained in the present study. In the current work, vitamin C showed an IC50 of (1.48 ± 0.03 mg/mL), reported a significantly lower IC50 value, indicating a higher free radical scavenging activity under their experimental conditions.

This variation in IC50 values suggests that the antioxidant efficiency of vitamin C may beinfluenced by differences in experimental design, reaction the intermediate maturity stage, and assay conditions.

In the study conducted by Hussain *et al*. (2025), vitamin C exhibited a strong free radical scavenging activity, with an IC_{50} value of 0.12 mg/mL and a high inhibition percentage reaching 97.3%, confirming its potent antioxidant efficiency. In comparison, the present study demonstrated that vitamin C recorded an IC_{50} value of 0.21 mg/mL, indicating a relatively lower antioxidant activity than that reported in the literature. Since IC_{50} values are inversely related to antioxidant potency, the increase in this value reflects a moderate reduction in free radical scavenging efficiency under the current experimental conditions. These findings suggest that although the tested sample exhibited appreciable antioxidant activity, its effectiveness remained lower than that of vitamin C as a standard reference antioxidant.

On the other hand, the study conducted by Cho *et al*. (2020) on the antioxidant and anti-inflammatory activities of *Capsicum annuum L*. reported that ascorbic acid (vitamin C) showed an IC_{50} value of 6.4 ± 0.9 mg/mL in the DPPH assay. In contrast, the present study revealed a markedly lower IC_{50} value for ascorbic acid (0.21 ± 0.04 mg/mL), indicating significantly higher antioxidant activity under the current experimental conditions.

In contrast, the study conducted by Lahbib *et al*. (2023) investigated the antioxidant activity of *Capsicum annuum* fruits across different ripening stages. Their results showed that antioxidant capacity was clearly influenced by the maturity stage, with the following IC_{50} values reported:unripe pepper: 59.28 ± 0.88, intermediate maturity stage: 70.76 ± 1.05, and ripe pepper: 76.72 ± 1.76. These finding sindicate a marked variation in antioxidant activity depending on the physiological stage of fruit development.

These results are consistent with those of the present study, where lower IC_{50} values were recorded for ripe peppe rcompared to the other stages, indicating higher free radical scavenging

activity with fruit maturation. However, a difference in the trend pattern can be observed between the two studies. Lahbib *et al*. (2023) reported a progressive increase in IC_{50} values from unripe pepper to ripe pepper, which may be attributed to differences in the accumulation of bioactive compounds or variations in cultivar characteristics and environmental conditions.

Accordingly, these discrepancies among studies can be explained by variations in experimental conditions, such as extraction methods, the type of solvent used, the plant material source, and the stage of maturity, in addition to assay protocols. These factors may significantly influence the assessment of antioxidant activity.

II.2.2 FRAP assay

The ferric-reducing antioxidant power, or FRAP assay, is a well-known technique for determining a substance's antioxidant potential. It works because antioxidants can change ferric ions (Fe3+) into ferrous ions (Fe2+).

The antioxidant activity of extracts from unripe pepper, pepper at the intermediate maturity stage, and ripe pepper was evaluated using the method illustrated in Figure (03)

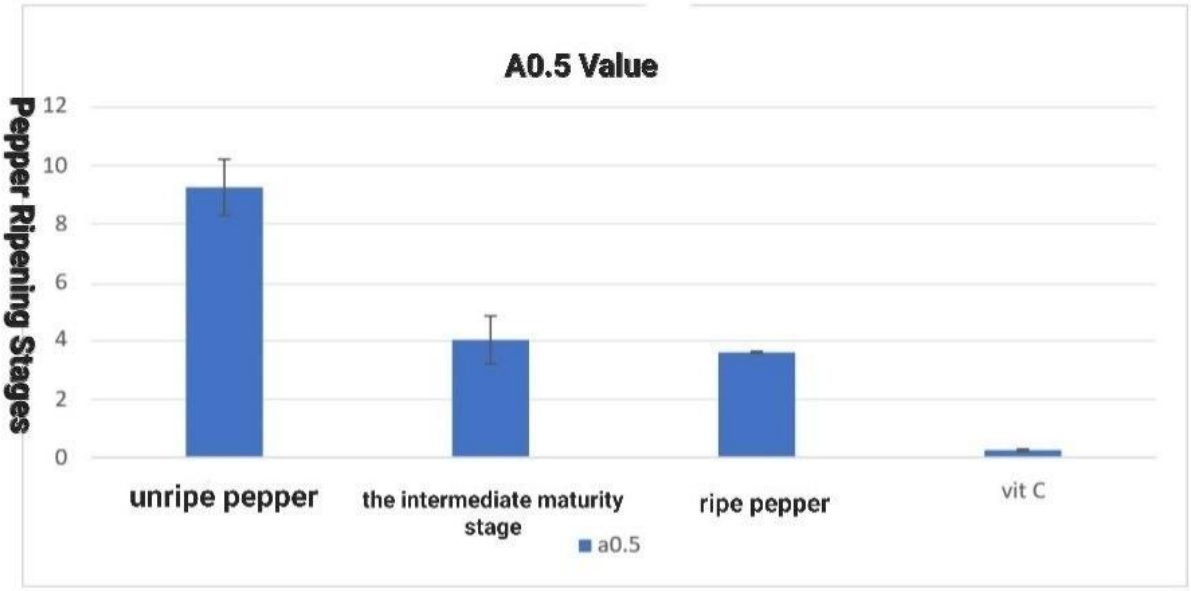

Figure II.3: Results of A0.5 values for the ferricreducing power assay

The results of the FRAP assay indicate a clear progression in antioxidant capacity across different ripening stages of the pepper extracts. The unripe pepper extract exhibited the highest FRAP value (9.28 ± 0.95), suggesting a comparatively weaker antioxidant activity among the tested pepper samples. This was followed by the intermediate maturity stage pepper extract with a A0.5 value of (4.04 ± 0.8). Notably, the ripe pepper extract demonstrated the lowest FRAP value (3.58 ± 0.04), indicating the strongest antioxidant activity. This trend suggests a

gradual improvement in the ferric reducing antioxidant power with increasing ripening stage, where more mature pepper sexhibit enhanced antioxidant capabilities.

Vitamin C demonstrated the strongest antioxidant activity in the FRAP assay, recording the lowest A0.5 value of 0.29 ± 0.04. This value reflects a markedly higher antioxidant activity capacity compared to the plant extracts investigated in this study.

In a study of Sadiq *et al*., (2025), vitamin C exhibited a lower half maximal inhibitory concentration value compared to that obtained in the present study. In the current work, vitamin C showed an half maximal inhibitory concentration of reported a significantly.An IC50 value of 1.48 ± 0.03 mg/mL was obtained, reflecting a markedly lower concentration and suggesting a superior antioxidant capacity with enhanced antioxidant activity under the tested experimental conditions .

In a recent study conducted by Momin Zarina *et al*. (2024), ascorbic acid (vitamin C), used as a standard antioxidant compound, demonstrated a strong antioxidant potential in the FRAP assay with an IC50 value of 3.503 µg/mL. The study confirmed that lower IC50 values are associated with greater ferric reducing antioxidant power and enhanced free radical scavenging efficiency.

In comparison, the present study revealed that ascorbic acid exhibited an IC50 value of 0.21 ± 0.04 mg/mL in the FRAP assay, indicating a considerable antioxidant activity under the current experimental conditions. Although differences in assay conditions, extract preparation, and concentration units may influence direct comparison, the obtained results still confirm the potent antioxidant capacity of vitamin C. These findings support the use of ascorbic acid as a reliable reference standard in antioxidant evaluation studies and demonstrate that the tested sample possesses appreciable reducing power, though its activity remains dependent on experimental parameters and assay sensitivity.

Akhter *et al*. (2024) observed that bioactive compounds and antioxidant activity in various chili cultivars in creased consistently with the stage of development. Their study reported FRAP values ranging from 142.62 to 311.03 mM Fe (II) Equivalent/100g DW, confirming that the ripening process is the primary driver of antioxidant accumulation

the investigated the phytochemical composition and antioxidant activity of elevenTunisian chili pepper varieties. Their results demonstrated that the ripening stage was the predominant factor influencing FRAP values, with a significant increase recorded from the green (unripe) to the red (ripe) stage. Specifically, Lahbib *et al*. (2023) reported FRAP levels ranging from 19.60 to 26.27 mmol TE g^{-1}, noting that the accumulation of antioxidant

precursors at advanced maturity stages facilitates the formation of diverse phenolic compounds with enhanced reducing capabilities.

Furthermore, Guilherme *et al*. (2020) provided a comparative analysis of green and red sweet peppers, highlighting that while green peppers may possess higher radical scavenging activity in certain assays like FRAP due to specific chlorophyll-related protectors, the total reducing capacity measured by FRAP often peaks in mature red fruits. This is attributed to the synthesis of secondary metabolites, such as carotenoids and specific flavonoids, which intensify during the final stages of maturation

Similarly, Ivan *et al*. (2024) characterized the antioxidant potential of *Capsicum annuum* extracts, reporting an EC50 value for FRAP of 0.561 mg/mL, which underscores the high efficiency of mature .pepper extracts in reducing ferric ions

The observed discrepancies in absolute values across different studies-such as those reported by Lahbib *et al*. (2023) versus the present findings-can be scientifically attributed to several factors.

These include variations in genotype (cultivar characteristics), environmental stressors (e.g., light exposure and temperature), and experimental protocols (e.g., solvent polarity and extraction efficiency). For instance, Lahbib *et al*. (2023) emphasized that fruits positioned in the upper parts of the plant, which receive higher solar radiation, exhibited significantly higher FRAP values compared to those in lower positions, suggesting that pre-harvest environmental conditions play a pivotal role .in modulating the antioxidant profile.

In conclusion, the progressive increase in FRAP activityduring maturation aligns with the metabolic shift toward the synthesis of bioactive compounds, confirming that ripe *Capsicum annuum* fruits serve as a superior .source of dietary antioxidants.

II.3 BSA test (Protein Denaturation Inhibition Assay)

The protein denaturation inhibition assay is a widelyused *in vitro* method for evaluating the anti-inflammatory potential of natural products and synthetic compounds. This assay is based on the principle that inflammation is associated with the denaturation of proteins, a process in which proteins lose their native structure under the influence of external stress such as heat or chemicals. Denatured proteins can trigger inflammatory responses by acting as autoantigens, there by promoting tissue damage. Substances capable of inhibiting protein denaturation are there fore considered to possess potential anti-inflammatoryactivity.

The anti -inflammatory activity of extracts from unripe pepper, pepper at the intermediate maturity stage, and ripe pepper was evaluated using the method illustrated in Figure (II.4)

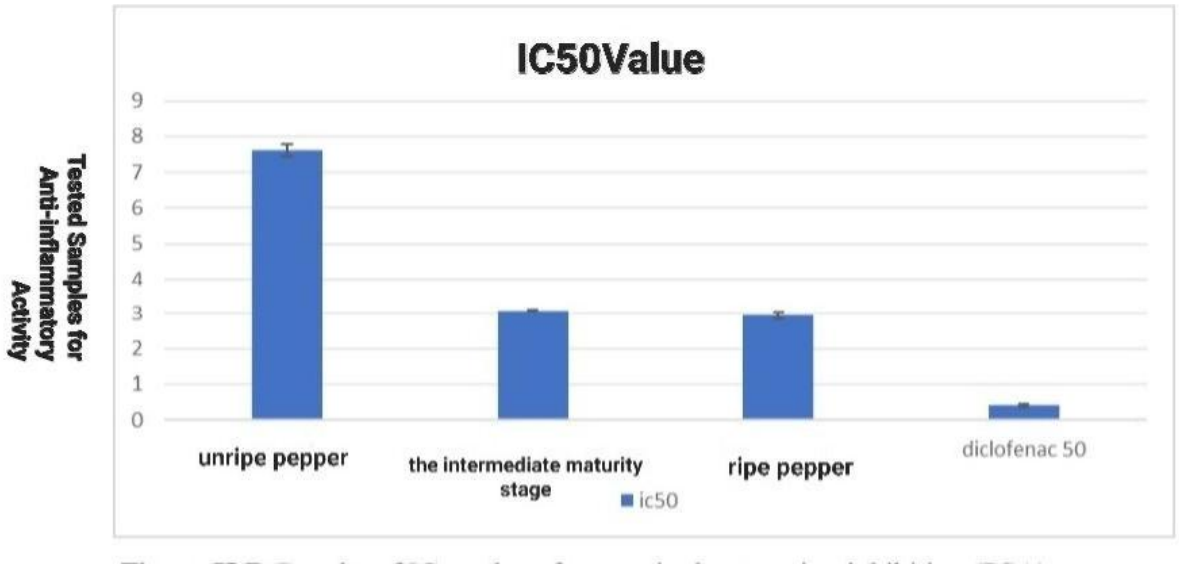

Figure II.4: Results of IC_{50} values for protein denaturation inhibition (PSA)assay

The IC_{50} values obtained for *Capsicum annuum* extracts ranged from 2.93 ± 0.05 to 7.62 ± 0.16, indicating a relatively strong inhibitory effect compared to several plant extracts described in previous studies. A study conducted in 2025 demonstrated that the extract of Averrhoa bilimbi showed an IC_{50} value of approximately 60.93 µg/mL, while Diclofenac sodium presented markedly stronger activity with a lower IC_{50} value of 8.20 µg/mL.

Similarly, Andrographis paniculata showed an IC_{50} of 14.14 ppm compared to 4.96 ppm for diclofenac, confirming the superior efficacy of the standard drug. Another study reported IC_{50} values of 180.70 µg/mL for plant extracts versus 29.45 µg/mL for diclofenac, while in Lavandulastoechas, values around 207 mg/mL were observed for both the extract and the reference drug, suggesting moderate activity depending on the phytochemical composition. In comparison, the relatively low IC_{50} values obtained in the present study demonstrate that *Capsicum annuum* extracts possess considerable anti-inflammatory potential, particularly in the advanced stage of fruit maturity. However, The activity is slower compared to the other groups. that of diclofenac sodium (0.40 ± 0.05), which is expected due to its well-established pharmacological potency. Overall, these findings support the hypothesis that the anti-inflammatory activity of *Capsicum annuum* is associated with its richness in bioactive compounds such as phenolics and flavonoids, which are known to inhibit protein denaturation and contribute to inflammation control

The anti-inflammatory potential of *Capsicum annuum* observed in the present study is in agreement with several recent investigations reporting protein denaturation inhibition (PSA) activity of the same species. In a study conducted by Rahman *et al*. (2021) on methanolic extracts of *Capsicum annuum* fruits, the IC_{50} values ranged from 5.10 to 9.45 , depending on maturity stage, which is comparable to the values obtained in the current work (2.93 ± 0.05 to

7.62 ± 0.16 µg/mL). This confirms that fruit developmental stage significantly influences anti-inflammatory efficiency, likely due to variations in phenolic accumulation.

Similarly, Kumar *et al*. (2022) reported moderate PSA inhibition activity in *Capsicum annuum* extracts with IC_{50} values around 8.30 µg/mL, which remains slightly higher than the most active fraction in the present study, suggesting stronger bioactivity in our tested extracts, possibly due to optimized extraction conditions or higher phytochemical yield.

In another comparative study, Oliveira *et al*. (2023) evaluated different Capsicum varieties and found IC_{50} values ranging from 6.80 to 12.50 µg/mL, indicating variability among cultivars. These results are still consistent with the relatively strong inhibition observed in the current study, particularly for mature fruits, which showed the highest activity.

Furthermore, Singh *et al*. (2024) highlighted that Capsicum-derived extracts exhibited PSA inhibition in the range of 4–10 µg/mL, attributing this effect to capsaicinoids, flavonoids, and total phenolic content. This supports the hypothesisthat the strong activity observed in the present work is mainly associated with the phytochemical richness of mature fruits.

When compared with standard anti-inflammatory drugs, such as diclofenac sodium (IC_{50} ≈ 0.3–1.0 µg/mL in recent assays), it is evident that the synthetic drug still exhibits superior potency. However, plant-based extracts offer a safer profile and multifunctional bioactivity, which increases their pharmacologica linterest.

II.2.3 Insecticide efficacy assay

Toxicity testing is used to evaluate the safety of natural or chemical substances and to determine their harmful effects on living organisms by studying toxic doses and their impact on vital functions. This test is essential to ensure the safe use of plant extracts and pesticides in agriculture and to reduce environmental and biological risks. As illustrated in the following Figure (II.5)

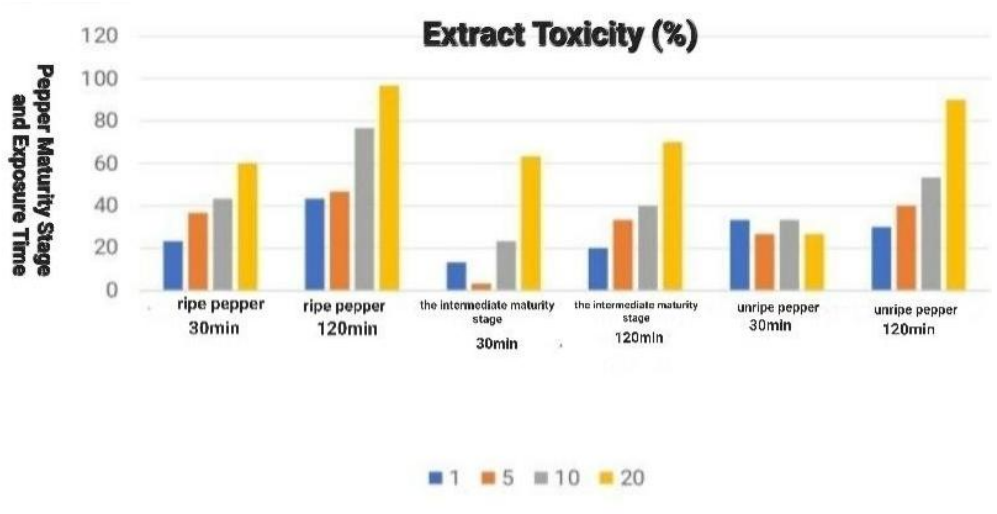

Figure II.5: Toxicity results of the extract

The results presented in the graph revealed a clear variation in the toxic effect or treatment efficacy according to pepper fruit size (unripe pepper, ripe pepper, the intermediate maturity stage), exposure time (30 and 120 min), and the tested concentrations (1, 5, 10, and 20). Overall, an increase in concentration resulted in a significant rise in the observed effect, as concentration 20 recorded the highest values in most treatments compared to the lower concentrations, indicating a positive correlation between concentration and the effectiveness of the tested extract or pesticide.

The findings also demonstrated that exposure time played an important role in enhancing the toxic activity, since the values obtained after 120 min were generally higher than those recorded after 30 min, particularly in ripe peppers and the intermediate maturity stage. This may be explained by the fact that longer exposure periods allow greater absorption and action of the active compounds on the target insect, thereby increasing the toxicity or inhibitory effect.

Regarding fruit size, ripe peppers exhibited the highest efficacy, especially at concentration 20 after 120 min, where the values approached 100%. This could be attributed to the higher content of biologically active compounds such as phenolics, flavonoids, and capsaicinoids, which are known for their insecticidal and toxic properties. In contrast, unripe peppers showed relatively lower efficacy at certain concentrations, which may reflect differences in their chemical composition or in the levels of secondary metabolites.

On the other hand, some lower concentrations produced weak responses after 30 min, particularly in the intermediate maturity stage peppers, suggesting that the toxic effect strongly depends on both dose and exposure duration. These findings are consistent with several studies reporting that plant extracts rich in phenolic compounds and carotenoids exhibit increased insecticidal activity with increasing concentration and exposure time.

Overall, these results confirm that pepper extracts possess considerable biological activity against the studied insect, and that this activity is influenced by several factors, including treatment concentration, exposure duration, and the size of the fruits used for extract preparation.

The results obtained in this study indicate that extracts of chili pepper (Capsicum spp.) exhibit a notable biological activity against aphids, affecting their survival rate, feeding behavior, and reproduction. A clear reduction in aphid populations was observed in treated groups compared to the control, with the absence of normal feeding activity, suggesting strong antifeedant and repellent effects.

These findings are consistent with those reported by Isman (2006), who demonstrated that plants rich in secondary metabolites such as capsaicinoids and alkaloids possess natural

insecticidal properties. These botanical insecticides act on the nervous and digestive systems of insects, leading to reduced feeding and increased mortality.

Similarly, Koul *et al*. (2008) reported that plant extracts containing phenolic compounds and terpenoids, including those from *Capsicum annuum*, can significantly disrupt the feeding behavior of aphids such as Aphis gossypii, resulting in a marked decrease in reproduction and fecundity, which supports the present findings.

In addition, Pavela (2015) demonstrated that capsaicin-containing plant extracts exhibit strong repellent activity against several aphid species, including Myzus persicae. These compounds interfere with the insect's nervous system and reduce its ability to ingest plant sap, thereby affecting its growth and development. Furthermore, Saxena (1989) reported that natural plant compounds act as feeding deterrents by interfering with insect chemoreception, which explains the reduced colonization of plants treated with chili pepper extracts.

Recent studies also indicate that capsaicin and its derivatives not only act as repellents but may also induce physiological disturbances in the insect central nervous system at higher concentrations, leading to increased mortality within 24–72 hours after exposure. Overall, these findings suggest that chili pepper extracts possess promising bioinsecticidal potential against aphids through multiple mechanisms, including repellency, feeding inhibition, and reduced reproduction, making them an environmentally friendly alternative to conventional chemical insecticides.

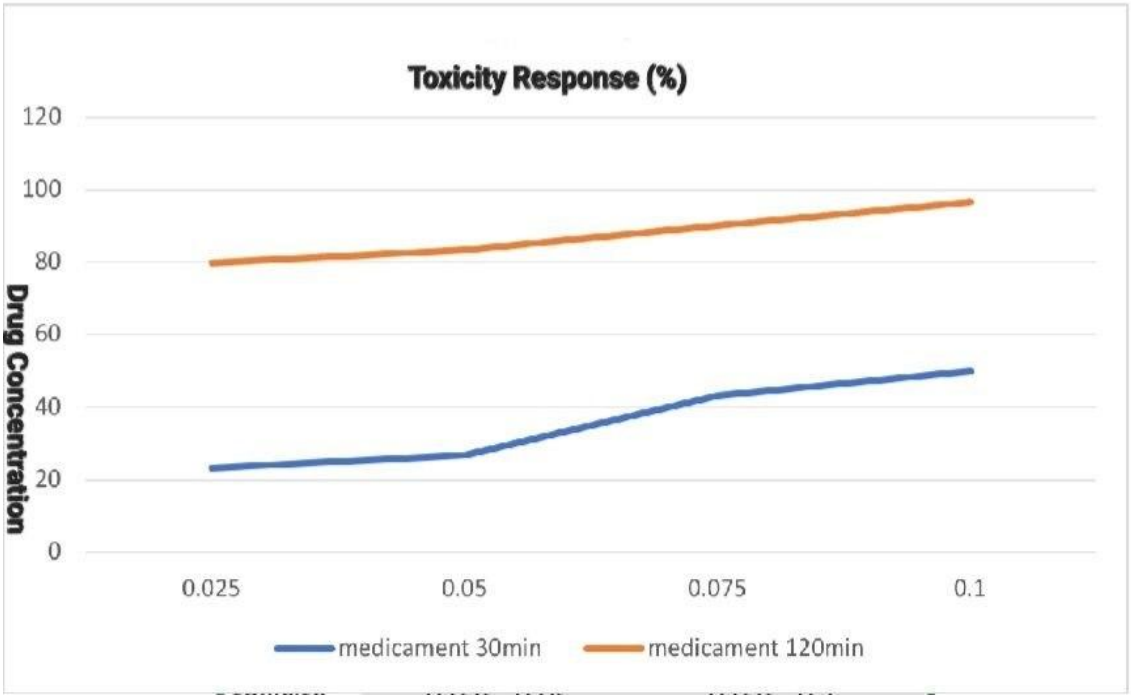

Figure II.6: Medication toxicity results

The graph (Figure II.6) represents the variation in the drug effect according to concentration and time (30 min and 120 min). It can be observed that the values increase progressively as the concentration rises from 0.025 to 0.1, indicating a positive correlation between drug concentration and its effect.

For the 30-minute curve (blue line), there is a noticeable increase in values from approximately 23 to 50, showing that the drug effect increases rapidly with higher concentrations over a short period of time.

For the 120-minute curve (orange line), the values remain higher than those of the 30-minute curve at all concentrations, increasing from about 81 to 97. This indicates that the drug effect becomes stronger after a longer exposure time.

A significant difference between the two curves can also be observed, suggesting that time plays an important role in drug efficacy, as the response after 120 minutes is greater than after 30 minutes.

The obtained results indicate a clear positive relationship between drug concentration and biological response, as the effect progressively increases with concentration from 0.025 to 0.1. This finding is consistent with the fundamental principles of pharmacology described by Katzung (2021), who reported that the dose–response curve increases with rising concentration until reaching a saturation level.

Furthermore, the results show that the drug effect is higher after 120 minutes compared to 30 minutes at all tested concentrations, indicating that exposure time plays an important role in enhancing drug efficacy. This is in agreement with Rowland & Tozer (2011), who demonstrated that pharmacological response depends not only on drug concentration but also on exposure time, where prolonged exposure may increase drug–receptor interaction or drug accumulation within the biological system.

In addition, the clear difference between the 30-minute and 120-minute curves supports the findings of Goodman & Gilman (2018), who emphasized that drug response is not only immediate but also influenced by the time-dependent distribution and action of the drug within the body.

These findings are further supported by recent pharmacological literature emphasizing the integrated role of concentration and exposure time in determining biological response. Gabrielsson *et al*. (2019) highlighted that dose–response–time relationships provide a more accurate representation of pharmacodynamic behavior. Similarly, Atkinson (2022) reported that pharmacological effects are strongly influenced by the full time-course of drug exposure, including distribution and delayed receptor interactions.

Moreover, Piotrowski & Buchanan (1982) demonstrated that both intensity and duration of exposure significantly affect biological response thresholds, while Morrison (1987) confirmed that time-dependent exposure leads to variations in observed pharmacological effects. These observations reinforce the importance of considering temporal dynamics in pharmacological evaluation.

Overall, these results are consistent with pharmacodynamic principles linking drug concentration, exposure time, and biological response, as described by Gabrielsson & Weiner (2007) and Derendorf & Meibohm (1999), who reported that increased exposure time enhances drug effect until a steady-state condition is reached.

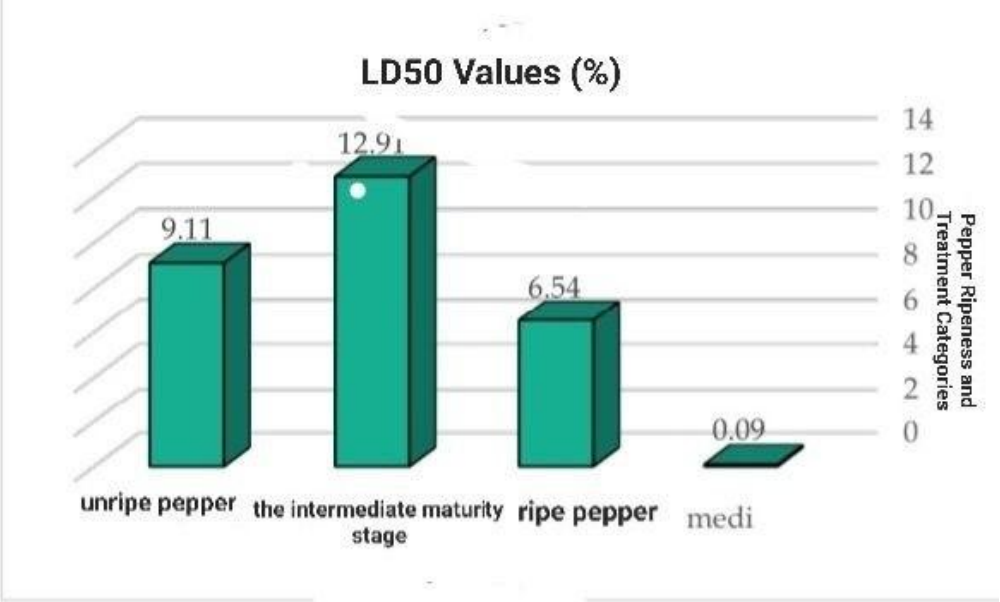

Figure II.7: Comparative Analysis of LD50 Values Across Different Pepper Size Categories and Medication Treatment

The bar chart representing the LD50 values demonstrates clear differences among the pepper samples according to their stage of ripeness. The unripe pepper group recorded an LD50 value of 9.11, whereas the ripe pepper group exhibited the highest value (12.91). In contrast, the fully ripe pepper group showed a lower value of 6.54, while the reference group (medi) displayed a very low value of 0.09. These variations indicate significant differences in the biological toxicity and physiological response among the tested pepper samples.

The elevated LD50 observed in the ripe pepper group may be attributed to a balanced concentration of bioactive compounds, particularly capsaicinoids and phenolic constituents, which could reduce the toxic effect compared with the other groups. Conversely, the lower LD50 value recorded for the fully ripe pepper group suggests a stronger toxic activity, possibly

due to higher concentrations or enhanced bioavailability of active compounds during advanced maturation stages.

These findings are consistent with the study conducted by Reyes-Escogido and colleagues (2011), who reported that capsaicinoid content varies depending on pepper maturity and directly affects biological activity and toxicity. Similarly, Bley *et al*. (2012) demonstrated that increasing concentrations of capsaicin may enhance toxic effects through its interaction with cellular membranes and metabolic pathways.

In addition, the observed variations may also be linked to differences in polyphenol and antioxidant contents among the pepper samples. Materska and Perucka (2005) confirmed that hot pepper fruits contain phenolic compounds with variable antioxidant and biological activities depending on cultivar, maturity stage, and fruit characteristics.

Overall, the results suggest that pepper ripening stage significantly influences LD50 values and biological toxicity. The ripe pepper sample exhibited the highest LD50 value, indicating lower relative toxicity, whereas the fully ripe pepper sample showed greater toxic activity compared with the other tested groups.

Conclusion

Conclusion

The present study provided a comprehensive biological and phytochemical investigation of *Capsicum annuum*, highlighting its richness in bioactive compounds and its multiple biological activities. Through the bibliographic and experimental approaches adopted in this work, it was demonstrated that chili pepper constitutes an important natural source of compounds with significant nutritional, pharmaceutical, and agricultural value.

The phytochemical analysis confirmed the presence of several major secondary metabolites, particularly polyphenols, flavonoids, and tannins which are recognized for their biological effectiveness. Quantitative determination of these compounds revealed that the extracts possess considerable amounts of phenolic constituents, suggesting a strong relationship between phytochemical composition and biological activity.

The evaluation of antioxidant activity using DPPH and FRAP assays demonstrated that the studied extracts exhibit an appreciable free radical scavenging capacity and reducing power. These antioxidant properties are mainly attributed to the high concentration of phenolic compounds and flavonoids capable of neutralizing reactive oxygen species and limiting oxidative stress. Furthermore, the anti-inflammatory activity assessed by the Protein Denaturation Inhibition Assay (BSA) showed promising inhibitory effects, indicating the potential therapeutic importance of chili pepper extracts in the management of inflammation-related disorders.

In addition, the insecticidal assay carried out against aphids revealed an interesting bioactivity of the extracts, suggesting their possible use as eco-friendly botanical insecticides. This biological effect may be related to the synergistic action of capsaicinoids and other secondary metabolites that interfere with the physiology and survival of insects. Such findings support the growing interest in developing natural alternatives to synthetic pesticides in sustainable agriculture.

Overall, the results obtained in this study confirm that *Capsicum annuum L*. represents a valuable source of biologically active compounds with antioxidant, anti-inflammatory, and insecticidal potential. These findings contribute to the valorization of medicinal and aromatic plants and encourage further investigations focusing on the isolation, characterization, and pharmacological evaluation of individual bioactive molecules. Future studies should also explore the mechanisms of action, toxicity profiles, and possible industrial applications of chili pepper extracts in the pharmaceutical, food, cosmetic, and agricultural sectors.

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الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

جامعة الشهيد حمه لخضر بالوادي

مركز تطوير المقاولاتية لجامعة الوادي

الكلية/ المعهد :علوم الطبيعة والحياة

القسم:بيولوجيا الخلوية والجزيئية

المستوى و التخصص :2ماستر بيوكيمياء

مذكرة التخرج لنيل شهادة جامعية – مشروع مؤسسة اقتصادية

عنوان المشروع:

مكافحة بيولوجية لمبيد حشري ضد الحشرات الزراعية من بينها حشرة المن

مقدمة في اطار الحصول على شهادة مؤسسة اقتصادية(مصغرة) في اطار القرار الوزاري 1275 المعدل و المتمم بالقرار الوزارى 008 المؤرخ فى 23 فيفرى 2025

Bioterra

شعار المؤسسة

السنة الجامعية: 2025 / 2026

الفهرس

1-فريق العمل

فريق المشروع	التخصص	الكلية/ المعهد	الخبرة/ الكفاءة في مجال المشروع
الطالب: هديل مدخل	بيوكيمياء	كلية علوم الطبيعة والحياة	تمتلك الطالبة معارف أكاديمية جيدة في مجال البيوكيمياء والمنتجات البيولوجية، إضافة إلى اهتمامها بالمجال الزراعي والبدائل الحيوية للمبيدات الكيميائية. كما تتميز بقدرتها على فهم طرق التحضير البيولوجي وتحليل المواد الحيوية المستعملة في مكافحة الحشرات الزراعية، مع مساهمتها الأساسية في تطوير فكرة المشروع والإشراف على الجانب العلمي والتقني الخاص بالمنتج.
الطالب: زينب شلبي	بيوكيمياء	كلية علوم الطبيعة والحياة	متلك الطالبة تكوينًا أكاديميًا في علوم البيوكيمياء والتحاليل المخبرية، مما يسمح لها بالمساهمة في مراحل التحضير، المراقبة المخبرية، ومتابعة جودة المنتج. كما تتميز بقدرتها على العمل الجماعي والمشاركة في الجوانب

			التنظيمية والتسويقية للمشروع
الطالب: قائد صفاء	بيوكيمياء	كلية علوم الطبيعة والحياة	تمتلك الطالبة معارف جامعية مرتبطة بالعلوم البيولوجية والتطبيقات البيئية، إضافة إلى اهتمامها بمجال المنتجات الطبيعية الصديقة للبيئة. وتساهم في متابعة مراحل التعبئة والتنظيم ودراسة السوق والتواصل مع الفئة المستهدفة من الزبائن.

2-فريق الإشراف

المشرف	التخصص	الكلية
المشرف الرئيسي : تليلي محمد العيد	بيولوجيا الخلوية والجزيئية	علوم الطبيعة والحياة
المشرف المساعد: رحيمة العيفة	محاسبة	كلية العلوم الاقتصادية والتجارة وعلوم التسير

ثانيا: تعريف المشروع: يكون النشاط وفقا للمدونات التي تحدد قائمة الأنشطة لكل هيئة تسجيل (المركز الوطني للسجل التجاري، الفلاحة، الصناعة التقليدية والحرف)؛

تحديد النشاط	قطاع النشاط	رمز النشاط (وفق مدونة الأنشطة للمركز الوطني للسجل التجاري cnrc)
في إنتاج **Bioterra** يتمثل نشاط مؤسسة مبيد حشري بيولوجي طبيعي موجه لمكافحة الحشرات الضارة بالمحاصيل الزراعية، خاصة الحشرات التي تؤثر على جودة وإنتاجية المنتوجات الزراعية بولاية الوادي. يعتمد المشروع على تصنيع منتج على شكل مسحوق (بودرة) يحتوي على مواد فعالة ذات أصل بيولوجي، بهدف	صناعي انتاجي	104211 صناعة مبيدات الحشرات والمنتجات المضادة للأمراض الفطرية والمعقمة. ويتضمن هذا النشاط تصنيع المنتجات المستعملة في مكافحة الحشرات والطفيليات والأمراض الزراعية، بما في ذلك المنتجات ذات المصدر الطبيعي أو البيولوجي.

توفير بديل آمن وصديق للبيئة مقارنة بالمبيدات الكيميائية التقليدية. يندرج المشروع ضمن قطاع الصناعات البيولوجية والزراعية الحديثة التي تهدف إلى دعم الفلاحة المستدامة وتقليل الأضرار البيئية والصحية الناتجة عن الاستعمال المكثف للمبيدات الكيميائية.		

❖ في حالة نشاط مقنن، أو نشاط يحتاج إلى اعتمادات او تراخيص خاصة، يجب ذكرها وذكر الجهة المانحة لها

نظرًا لأن المشروع مرتبط بإنتاج مبيدات حيوية موجهة للاستعمال الزراعي، فإنه قد يتطلب:

.اعتماد من المصالح المختصة بوزارة الفلاحة والتنمية الريفية

.مطابقة المعايير الصحية والبيئية الخاصة بالمنتجات البيولوجية

.الحصول على شهادة مطابقة من الهيئات المعتمدة الخاصة بمراقبة الجودة

.تسجيل المنتج وفق التنظيمات المعمول بها في الجزائر المتعلقة بالمبيدات والمنتجات الزراعية

ثالثا: وصف فكرة المشروع:

ينغي ان يكون صاحب / أصحاب المشروع على دراية بكل تفاصيل المشروع وقادرين على تقديم وصف ملخص ودقيق عنه ، من خلال الإجابة بدقة على العناصر التالية :

ما هي منتجاتي أو خدماتي: (حدد بدقة منتجاتك. خدماتك):

في إنتاج مبيد حشري بيولوجي طبيعي على شكل مسحوق (بودرة)، موجه لمكافحة **Bioterra تتمثل منتجات مؤسسة**

.الحشرات الزراعية التي تصيب المحاصيل الفلاحية بولاية الوادي والمناطق المجاورة

يعتمد المنتج على مواد فعالة طبيعية ذات خصائص بيولوجية تساعد على الحد من انتشار الحشرات الضارة دون التسبب

.في أضرار كبيرة على البيئة أو صحة الإنسان، بعكس المبيدات الكيميائية التقليدية

:ويهدف المشروع إلى تطوير منتج محلي فعال يتميز بـ

.تقليل التأثيرات البيئية السلبية

.المحافظة على جودة التربة والمحاصيل الزراعية

.المساهمة في تطوير الفلاحة المستدامة

.توفير بديل بيولوجي آمن للفلاحين

دعم التوجه نحو المنتجات الزراعية الصحية والصديقة للبيئة

من سيشتري منتجاتي/ خدماتي (حدد زبائنك بدقة) :

تستهدف المؤسسة عدة فئات من الزبائن، أهمها:

.الفلاحون وأصحاب المستثمرات الزراعية بولاية الوادي

.منتجو الخضر والفواكه والمحاصيل الزراعية

.التعاونيات الفلاحية

.محلات بيع المنتجات الزراعية والأسمدة

.المؤسسات الناشطة في المجال الزراعي

.المستثمرات المهتمة بالفلاحة البيولوجية

كما يستهدف المشروع الفلاحين الذين يسعون إلى تقليل الاعتماد على المبيدات الكيميائية وتحسين جودة منتجاتهم الزراعية بما يتوافق مع متطلبات السوق الحديثة

أين سيقام مشروعي وكيف تصل منتجاتي / خدماتي للزبائن :(بين مكان إقامة مشروعك و طرق التواصل مع زبائنك)

يقام المشروع بولاية الوادي، باعتبارها من الولايات ذات النشاط الفلاحي الكبير في الجزائر، وخاصة في مجال زراعة الخضر والنخيل والمحاصيل الزراعية المختلفة، مما يجعل الطلب على وسائل مكافحة الحشرات مرتفعًا ومستمرًا.

:وسيتم توزيع المنتجات عبر

.البيع المباشر للفلاحين

.التعاقد مع محلات بيع المستلزمات الزراعية

.التسويق عبر وسائل التواصل الاجتماعي

.المشاركة في المعارض والتظاهرات الفلاحية

التوصيل المباشر للمستثمرات الزراعية الكبرى

كيف سيشتغل مشروعي (بين من سيقوم بتشغيل المشروع ومن سيساهم في ذلك):

سيعتمد المشروع على فريق عمل متخصص في مجال البيوكيمياء والإنتاج الحيوي، حيث يتم تحضير المادة الفعالة البيولوجية وفق خطوات إنتاج مخبرية وصناعية دقيقة، ثم تحويلها إلى منتج نهائي على شكل مسحوق جاهز للاستعمال.

:تمر عملية الإنتاج بعدة مراحل، أهمها

.تحضير المواد الأولية

.المعالجة البيولوجية واستخلاص المادة الفعالة

.التجفيف والطحن

.تعبئة المنتج وتغليفه

.مراقبة الجودة

.التخزين والتوزيع

كما سيساهم المشروع في توفير مناصب شغل مباشرة وغير مباشرة في المجال الزراعي والصناعي

مبررات اختيارفكرة مشروعي (اشرح لماذا تم اختيار هذه الفكرة بالذات):

تم اختيار هذه الفكرة لعدة أسباب علمية واقتصادية وبيئية، أهمها:

.الانتشار الكبير للحشرات الزراعية التي تؤثر سلبًا على المحاصيل الفلاحية بولاية الوادي

.زيادة الطلب على المبيدات البيولوجية الصديقة للبيئة

.ارتفاع الوعي بأضرار المبيدات الكيميائية على صحة الإنسان والتربة

.توفر البيئة الزراعية المناسبة لإنجاح المشروع

.انسجام المشروع مع التوجهات الحديثة نحو الفلاحة المستدامة

.إمكانية تطوير المنتج محليًا باستعمال معارف علمية مكتسبة في تخصص البيوكيمياء

مساهمة المشروع في دعم الاقتصاد المحلي وتقليل استيراد بعض المنتجات الزراعية الكيميائية

رابعا: دراسة السوق

1. **طبيعة وشدة المنافسة:** من هم المنافسون المباشرون وغير المباشرون؟

المنافسون	طبيعة المنافسة		شدة المنافسة		
	مباشرة	غير مباشرة	قوية	متوسطة	ضعيفة
شركات بيع المبيدات الكيميائية الزراعية	X		X		
محلات بيع المنتجات الزراعية والأسمدة	X			X	
المنتجات البيولوجية المستوردة	X			X	

أذكر استراتيجيات مؤسستك في التعامل مع منافسيها:

على مجموعة من الاستراتيجيات التنافسية، أهمها **Bioterra** ستعتمد مؤسسة

.تقديم منتج بيولوجي آمن وصديق للبيئة

.التركيز على الجودة والفعالية في مكافحة الحشرات

.اعتماد أسعار تنافسية مقارنة بالمنتجات المستوردة

.بناء علاقة ثقة مع الفلاحين من خلال التوعية والإرشاد

.استهداف المستثمرات الزراعية الحديثة المهتمة بالفلاحة النظيفة

.التسويق الرقمي عبر منصات التواصل الاجتماعي

.المشاركة في المعارض والصالونات الفلاحية

تطوير المنتج مستقبلاً بما يتناسب مع احتياجات السوق المحلي

2. **حجم السوق المستهدف والمحتمل:**

- **حجم السوق المستهدف:** يمثل مجموعة الافراد أو المؤسسات والتي تقدم لها او تعرض عليها – منتجاتك/خدامتك. (يطلب تحديد الشريحة المستهدفة، تعدادها، مكان تواجدها، .....).
- يتمثل السوق المستهدف لمؤسسة **Bioterra** في الفلاحين والمستثمرات الزراعية بولاية الوادي، خاصة الناشطين في:

- زراعة الخضر المحمية.
- زراعة النخيل والتمور.
- الزراعات الموسمية.
- المستثمرات الفلاحية الكبرى.
- التعاونيات الفلاحية.
- وتعد ولاية الوادي من أهم الولايات الزراعية في الجزائر، حيث تشهد توسعًا كبيرًا في النشاط الفلاحي، مما يؤدي إلى ارتفاع الحاجة إلى منتجات مكافحة الحشرات الزراعية بشكل مستمر.
- كما يستهدف المشروع الفلاحين الباحثين عن حلول بديلة للمبيدات الكيميائية بهدف تحسين جودة الإنتاج الزراعي والحفاظ على البيئة والصحة العامة
- **حجم السوق المحتمل**: السوق المحتمل هو مجموعة الافراد او المؤسسات التي تطلب أو يحتمل ان تطلب منتجاتك او خدماتك لإشباع حاجا تهم ورغباتهم. (من يشتري منتجاتنا/ خدماتنا؟ من وما الذي يحفزه على ذلك؟ أين يتواجدون؟ كم أعدادهم؟).
- يشمل السوق المحتمل جميع الفلاحين والمؤسسات الزراعية داخل ولاية الوادي والولايات المجاورة التي يمكن أن تعتمد مستقبلاً على المبيدات البيولوجية كبديل للمبيدات الكيميائية.
- ويتميز هذا السوق بإمكانيات نمو كبيرة نتيجة:
- التوجه العالمي نحو المنتجات البيولوجية.
- ارتفاع الوعي البيئي والصحي.
- تطور قطاع الفلاحة المستدامة.
- زيادة الطلب على المنتجات الزراعية الصحية.
- تشجيع الدولة للمشاريع البيئية والابتكارية.
- كما يمكن للمؤسسة مستقبلاً توسيع نشاطها نحو ولايات أخرى ذات نشاط فلاحي مكثف، مثل:
- بسكرة.
- ورقلة.
- تقرت.
- المغير.

3. **تحليل SWOT:** نقاط القوة والضعف والفرص والتهديدات لمشروعي:

- **نقاط القوة:** كل العناصر (داخل المؤسسة) التي تشكل مصدر قوة لمشروعي (قد تكون: مهارات خاصة، موارد بشرية، موارد مادية، .....)
- **نقاط الضعف:** كل العناصر (داخل المؤسسة) التي تشكل نقطة ضعف أو تقف عائقا امام مشروعي حاليا أو مستقبليا
- **الفرص:** كل العناصر الخارجية التي لها أثر إيجابي على تنفيذ مشروعي أو تطوره في المستقبل
- **التهديدات:** كل العناصر الخارجية التي لها أثر سلبي على تنفيذ مشروعي أو تطوره في المستقبل

جدول تحليل SWOT

SWOT	عوامل تساعد في تحقيق الأهداف	عوامل تعطل تحقيق الأهداف

	نقاط القوة Strengths	نقاط الضعف Weaknesses
عوامل داخلية	1 شروع مبتكروصديق للبيئة 2 تخصص الفريق في البيوكيمياء 3 تو افق المشروع مع الفلاحة المستدامة 4 توفر الطلب على منتجات مكافحة الحشرات	1 نقص الخبرة الميدانية في التسيير الصناعي. 2 محدودية الإمكانيات المالية في البداية. 3 ضعف شهرة العلامة التجارية في بداية النشاط. 4 الحاجة إلى تراخيص واعتمادات تنظيمية
عوامل خارجية	الفرص Opportunities 1 تزايد الوعي بأضرار المبيدات الكيميائية 2 دعم الدولة للمشاريع البيئية والابتكارية. 3 توسع النشاط الفلاحي بولاية الوادي 4إمكانية التوسع نحو أسواق جديدة	التهديدات Threats 1 المنافسة القوية من المبيدات التقليدية. 2 تقلب أسعار المواد الأولية. 3 صعوبة إقناع بعض الفلاحين بالمنتجات البيولوجية في البداية 4 احتمال ظهور منتجات منافسة مستوردة

4. نموذج العمل التجاري BMC لمشروعي:

<table>
<tr>
<td rowspan="2">العملاء
- الفلاحون.
- المستثمرات الزراعية.
- التعاونيات الفلاحية.
- محلات بيع المنتجات الزراعية.
- المؤسسات المهتمة بالفلاحة البيولوجية</td>
<td>العلاقة مع العملاء
- المتابعة المباشرة للفلاحين.
- تقديم الإرشادات والنصائح الزراعية.
- خدمات ما بعد البيع.
- التواصل عبروسائل التواصل الاجتماعي.
- المشاركة في المعارض الفلاحية.</td>
<td rowspan="2">القيمة المقترحة
- مبيد حشري طبيعي وآمن.
- منتج صديق للبيئة.
- تقليل الأضرار الصحية للمبيدات الكيميائية.
- المحافظة على جودة التربة والمحاصيل.
- سعر تنافسي مقارنة بالمنتجات المستوردة.
- دعم الفلاحة المستدامة</td>
<td>الأنشطة الرئيسية
- تصنيع المبيد البيولوجي.
- مراقبة الجودة والتحاليل المخبرية.
- التعبئة والتغليف.
- التسويق والترويج للمنتج.
- التوزيع والبيع.
- تطوير وتحسين المنتج</td>
<td rowspan="2">الشركاء الرئيسيون
- موردي المواد الأولية الطبيعية.
- المخابر الجامعية ومراكز البحث العلمي.
- مديرية الفلاحة.
- التعاونيات الفلاحية.
- محلات بيع المستلزمات الزراعية.
- هيئات دعم المؤسسات الناشئة.
- مؤسسات النقل والتوزيع.</td>
</tr>
<tr>
<td>قنوات توزيع
- البيع المباشر.
- محلات بيع المستلزمات الزراعية.
- المعارض والصالونات الفلاحية.
- التسويق الرقمي.
- التوصيل للمستثمرات الكبرى</td>
<td>الموارد الرئيسية
- الخبرة العلمية للفريق.
- المواد الأولية البيولوجية.
- معدات التحضير والتعبئة.
- Bioterra العلامة التجارية.
- المختبر والتجهيزات التقنية</td>
</tr>
</table>

الإيرادات	التكاليف
- بيع المبيد البيولوجي.	- شراء المواد الأولية.
- عقود التوريد مع المستثمرات الفلاحية.	- تكاليف التجهيزات والمعدات.
- التوزيع بالجملة لمحلات المنتجات الزراعية.	- أجور العمال.
- التوسع نحو منتجات بيولوجية مستقبلية	- تكاليف التسويق.
	- النقل والتوزيع.
	- تكاليف التراخيص والاعتمادات.
	- تكاليف الكهرباء والماء والصيانة

خامسا: الدراسة التقنية

1. **احتياجات المشروع من الموارد البشرية:** حدد بدقة احتياجات مشروعك من الموارد البشرية

طبيعة المستخدمين	العدد	الكفاءات / الشهادات
المسير	1	ماستر بيولوجيا شهادة جامعية في البيوكيمياء أو إدارة المشاريع مع القدرة على التسيير والمتابعة
الإطارات (مهندسين، رؤساء المصالح، المكلفين بالتسويق ، رؤساء الورشات...إلخ.)	1	المكلف بالتسويق - مختص في البيوكيمياء والتحاليل المخبرية. - مكلف بالتسويق والمتابعة التجارية.
عمال التحكم (المكلفين بالمخزن، السكريتاريا...إلخ.)	1	المكلف بالمخزن - مراقبة التعبئة والتغليف. - تسيير الوثائق الإدارية.
عمال التنفيذ (السائق، الحارس، عامل النظافة...إلخ.)	1	عامل نظافة - شغيل معدات الإنتاج. - التعبئة والتغليف. - النقل والتنظيف

2. **اختيار موقع إقامة المشروع : أين سيكون موقع المشروع ومبررات اختياره ؟** (قدم المبررات اللازمة لاختيار موقع المشروع)

سيقام المشروع بولاية الوادي، باعتبارها من أهم الولايات الزراعية في الجزائر، حيث تشهد نشاطًا فلاحيًا واسعًا خاصة في مجال زراعة الخضر المحمية والنخيل. ويتميز الموقع بعدة عوامل تساعد على نجاح المشروع، أهمها:

- ارتفاع الطلب على منتجات مكافحة الحشرات الزراعية.
- قرب المشروع من الفلاحين والأسواق المستهدفة.
- توفر بيئة مناسبة لتجريب وتطوير المنتج.
- سهولة الوصول إلى المستثمرات الزراعية.
- انخفاض تكاليف النقل مقارنة بالإنتاج خارج المنطقة.
- مساهمة المشروع في دعم التنمية المحلية وخلق مناصب شغل

سادسا: تقدير تكاليف و إيرادات المشروع

1 - تقدير تكاليف المشروع

1.1 تقدير المبلغ الإجمالي للاستثمار: (تكلفة المشروع)

البيان	المبلغ (دج)
آلات ومعدات الإنتاج	6.000.000
تهيئة المحل	25.0000
الأصول البيولوجية (الحيوانات- الأشجار...)	100.000
العتاد المتنقل	2500.000
العتاد المكتبي	120 .000
عتاد الإعلام الآلي	180 .000
عتاد السمعي البصري	0
عتاد الاتصالات	40.000
تركيب التجهيزات	300.000
المصاريف العامة المتعلقة بالإنشاء	160.000
رأس المال العامل (المادة الأولية)	500.000
المبلغ الإجمالي للاستثمار (تكلفة المشروع)	10.150.000

2.1 تقدير مشتريات المشروع من المواد الأولية

قائمة المواد الأولية		السنة 1	السنة 2	السنة 3
المادة الاولية 1: الفلفل	الكمية المشترات	1.600كغ	3.000كغ	5.000 كغ
	سعر الوحدة	150دج/كغ	170 دج/كغ	200دج/كغ
	تكلفة شراء المادة الاولية 1	240.000دج	510.000دج	1.000.000دج
المادة الاولية 2: المواد الحافظة	الكمية المشترات	80 كغ	150كغ	250كغ
	سعر الوحدة	1.000دج/كغ	1.100دج/كغ	1.200دج/كغ
	مبلغ المادة الاولية 2	80.000دج	165.000دج	300.000دج

المادة الاولية 3: أكياس تعليب	الكمية المشترات	8.000كيس	15.000كيس	25.000كيس
	سعر الوحدة	25دج	27دج	30دج
	تكلفة شراء المادة الاولية 3	200.000دج	405.000دج	750.000دج
إجمالي مبالغ المشتريات من المواد الاولية		520.000دج	1.080.000دج	2.050.000دج

3.1 – تقدير تكاليف اليد العاملة

تسمية المنصب	عدد العمال			قيمة الاجر		
	السنة 1	السنة 2	السنة 3	السنة 1	السنة 2	السنة 3
المسير/ أو المسيرون	1	1	1	600.000	600.000	600.000
الإطارات (مهندسين، رؤساء المصالح، المكلفين بالتسويق ، رؤساء الورشات...إلخ.)	1	1	1	540.000	540.000	540.000
عمال التحكم (المكلفين بالمخزن، السكريتاريا...إلخ.)	1	1	1	420.000	420.000	420.000
عمال التنفيذ (السائق، الحارس، عامل النظافة...إلخ.)	1	1	1	360.000	360.000	360.000

- ينبغي ان لا يقل الاجر الشهري الصافي عن الحد الأدنى المضمون

4 .1 تقدير التكاليف الخارجية للمشروع

البيان	السنة 01	السنة 02	السنة 03
مصاريف الكهرباء /الغاز /الماء	120.000	150.000	180.000
تكاليف الصيانة و التصليح	80.000	150.000	150.000
تكاليف الايجار	300.000	300.000	300.000
مصاريف تامين العتاد و المعدات	50.000	60.000	80.000
مصاريف التسويق (الدعاية والإشهار)	150.000	220.000	300.000
تكاليف التكوين والتدريب	30.000	40.000	50.000
مصاريف عامة (أعباء خارجية) أخرى	70.000	100.000	120.000
المجموع	800.000 دج	970.000 دج	1.180.000 دج

5 .1 تقدير تكاليف اهتلاك الاستثمارات

البيان	مدة الاهتلاك (بالسنوات)	قسط الاهتلاك السنوي (دج)
آلات ومعدات الإنتاج	10	600.000
العتاد المتنقل	5	500.000
العتاد المكتبي	10	12.000

عتاد الإعلام الآلي	3	60.000
عتاد السمعي البصري	\	\
عتاد الاتصالات	5	8.000
تركيب التجهيزات	10	30.000
المبلغ الإجمالي		**دج 1.210.000**

- يطبق الاهتلاك الخطي (تحدد مدة الاهتلاك وفقا لقرار وزارة المالية المؤرخ في 14 أفريل 2025 المعدل والمتمم للقرار الصادر بتاريخ 25 فيفري 2024 الذي يحدد مدة اهتلاك التثبيتات المطبقة لتحديد النتيجة الجبائية – الجريدة الرسمية عدد 29 لسنة 2025)

3-تقدير إيرادات المشروع

1.3 تقدير حجم المبيعات السنوية

البيان		السنة 1	السنة 2	السنة 3
المنتج / الخدمة 1:	الكمية المباعة	8.000	15.000	25.000
	سعر الوحدة	دج 1.000	دج 1.000	دج 1.000
	قيمة مبيعات المنتج 1	دج 8.000.000	15.000.000	25.000.000
المنتج / الخدمة 2:	الكمية المباعة			
	سعر الوحدة			
	قيمة مبيعات المنتج 2			
المنتج / الخدمة 3:	الكمية المباعة			
	سعر الوحدة			
	قيمة مبيعات المنتج 3			
إجمالي المبيعات				

2.3 حساب النتيجة السنوية

البيان	السنة 01	السنة 02	السنة 03
رقم الأعمال (المبيعات) ... (1)	8.000.000	15.000.000	25.000.000
المشتريات المستهلكة ... (2)	520.000	1.080.000	2.050.000
الأعباء الخارجية ... (3)	800.000	970.000	1.180.000
القيمة المضافة ... (4)= (1)-(2)-(3)	6.680.000	12.950.000	21.770.000
الأجور و الأعباء الاجتماعية ...(5)	1.920.000	2.280.000	2.640.000

إجمالي فائض الاستغلال ... (6) = (4)-(5)	4.760.000	10.670.000	19.130.000
الإهتلاكات و المؤونات....(7)	1.210.000	1.210.000	1.210.000
ناتج الاستغلال..... (8) = (6) - (7)	3.550.000	9.460.000	17.920.000
الضرائب على الشركات/ IFU .... (9)	400.000	750.000	1.250.000
صافي الدخل ..... (10) = (8) - (9)	3.150.000 دج	8.710.000 دج	16.670.000 دج
تطوررقم الأعمال	/	**+87.5%**	**+66.67%**

خاتمة:

القيمة المضافة المتوقعة للمشروع على الاقتصاد المحلي والوطني.

يعد مشروع Bioterra من المشاريع الابتكارية التي تجمع بين البعد الاقتصادي والبيئي، حيث يهدف إلى إنتاج مبيد حشري بيولوجي آمن وفعال يساهم في حماية المحاصيل الزراعية والحد من الآثار السلبية للمبيدات الكيميائية. كما ينسجم المشروع مع التوجهات الحديثة نحو الفلاحة المستدامة والتنمية البيئية

ومن المتوقع أن يساهم المشروع في:

-دعم الاقتصاد المحلي.

-خلق فرص عمل جديدة.

-تشجيع استعمال المنتجات البيولوجية.

-تحسين جودة الإنتاج الزراعي.

-تقليل التلوث البيئي.

- دعم الابتكار في القطاع الفلاحي الجزائري

كما يمتلك المشروع إمكانيات جيدة للتوسع مستقبلاً نحو أسواق جديدة وتطوير منتجات بيولوجية أخرى تخدم القطاع الزراعي الوطني.