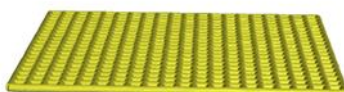


**Etude du potentiel herbicide des extraits
végétaux des espèces xero-halophytes Tunisiennes
et détermination de leurs modes d'action**



Promoteurs : Pr Haissam Jijakli – Pr Riadh Ksouri

Année civile : 2020

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE - GEMBLoux AGRO-BIO TECH

**Etude du potentiel herbicide des extraits végétaux des
espèces xero-halophytes Tunisiennes et détermination de
leurs modes d'action**

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Dissertation originale présentée en vue de l'obtention du grade de
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Promoteurs : Pr Haïssam Jijakli – Pr Riadh Ksouri

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Résumé

Les adventices constituent une contrainte majeure pour l'agriculture. En effet, connues par leur pouvoir allélopathique important, ces adventices peuvent causer des dommages économiques sévères atteignant 34% dans les grandes cultures. Dans cette optique, suite à la forte limitation des herbicides chimiques sur le marché et plus spécifiquement le retrait progressif du glyphosate, la demande du public pour des herbicides alternatifs d'origine biologique et davantage respectueux de l'environnement, est croissante. Cette thèse s'inscrit dans la politique de minimiser l'utilisation des herbicides chimiques principalement le glyphosate. Les travaux de recherche effectués dans le cadre de cette thèse ont permis de sélectionner 2 extraits végétaux à forte activité herbicide à savoir, l'extrait phénolique de *Cynara cardunculus* et l'huile essentielle de *Rosmarinus officinalis*. Par la suite nous avons procédé à un fractionnement bio-guidé afin de déterminer les composés bioactifs majoritaires. Ainsi, la myricitrine extraite de *C. cardunculus* a été identifiée pour la première fois comme molécule à potentiel herbicide. Ensuite, nous avons élaboré une nouvelle formulation à base de ces deux extraits végétaux sélectionnés qui pour la première fois améliore en même temps leurs efficacités et leurs stabilités. Finalement, l'étude des modes d'action de l'extrait phénolique de *C. cardunculus* a été réalisé par l'analyse des paramètres physiologiques et biochimiques de la plante cible traitée par nos deux extraits végétaux et en même temps par l'étude de l'interaction des molécules bioactives avec la membrane lipidique de *A. thaliana*.

Abstract

Weeds are a major constraint for agriculture. Indeed, known by their significant allelopathic power, these weeds can cause severe economic damages up to 34% in field crops. In this context, following the strong limitation of chemical herbicides on the market and more specifically the gradual withdrawal of glyphosate, the public demand for alternative herbicides of biological origin and more respectful to the environment, is increasing. This thesis is part of the policy of minimizing the use of chemical herbicides, mainly glyphosate. The research work carried out in the framework of this thesis allowed the selection of 2 plant extracts with high herbicidal activity, namely the phenolic extract of *C. cardunculus* and the

essential oil of *R. officinalis*. Subsequently, we proceeded to a bio-guided fractionation to determine the majority bioactive compounds. Indeed, myricitrin extracted from *C. cardunculus* has been identified for the first time as molecules with herbicidal potential. Then, we have also developed a new formulation based on these two selected plant extracts which for the first time improves both their effectiveness and their stability. Finally, we studied the modes of action of the phenolic *C. cardunculus* extract. This was done by analyzing the physiological and biochemical parameters of the target plant treated by our two plant extracts and at the same time studying the interaction of bioactive molecules with the lipid membrane of *A. thaliana*.

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Liste des abréviations

HPLC: high pressure liquid chromatography

GC-MS: gas chromatography mass spectrometry

CO₉: Ethoxylated (9) Castor oil

MDA: malondialdehyde

PPM: plant plasma membrane

EO: essential oil

ROS: reactive oxygen species

ADN: acide désoxyribonucléique

PDA: Potato dextrose agar

PDB: Potato dextrose broth

ml: milliliter

Elisa: Enzyme-Linked Immunosorbent Assay

μl: microliter

MeOH: methanol

DW: dry weight

MS : Métabolites secondaires

HE : huiles essentielles

SCE : extraction super critique

SFE : extraction de fluide supercritique

PAM : plantes aromatiques et médicinales

MDA : malondialdehyde

PSII : photosystème II

PCD : programmed cell death

Introduction générale

Les méthodes alternatives (thermiques, mécaniques) proposées actuellement pour lutter contre les adventices s'avèrent peu efficaces dans le temps. Ainsi, les agriculteurs ont toujours recours à l'utilisation des herbicides traditionnels chimiques qui peuvent être nocifs pour l'environnement et la santé humaine. Pourtant, la demande du public pour des herbicides alternatifs, d'origine biologique (bioherbicide) et davantage respectueux pour l'environnement est croissante. Dans cette optique, notre recherche s'inscrit dans le cadre de la politique de réduction du nombre de produits phytosanitaires autorisés en Europe et plus particulièrement dans le cadre du retrait du glyphosate (matière active la plus vendue actuellement au niveau mondial, avec un marché estimé à 27 milliards d'euros, est considérée potentiellement cancérigène). Dans un deuxième temps, les extraits végétaux y compris les huiles essentielles ont été étudiés depuis de nombreuses années pour leurs propriétés fongicides et bactéricides (en médecine humaine et vétérinaire et plus récemment en protection des cultures). Très récemment, certaines publications ont montré le potentiel bioherbicide de certains extraits végétaux en conditions contrôlées. Il s'agit d'études limitées tant du point de vue des extraits végétaux testés que du nombre et de la diversité des adventices sur lesquelles ils ont été testés. De plus, l'analyse de l'état de l'art montre qu'aucune formulation n'a pour l'instant été développée et que les modes d'action qui permettraient l'élaboration d'une telle formulation sont méconnus. Pour cette raison la présente thèse s'intéresse à l'étude du potentiel herbicide des extraits végétaux et à l'étude du mode d'action de leurs composés actifs identifiés. Le chapitre 1 est une introduction bibliographique qui met en évidence la problématique des adventices en agriculture, les méthodes de lutte actuelles et l'utilisation des composés allélochimiques pour faire face à cette problématique. Le chapitre 2 se focalise sur les objectifs de la thèse, la stratégie suivie (en particulier par rapport aux choix des plantes, des adventices et des molécules naturelles). D'autre part, les résultats obtenus dans ce travail sont présentés sous forme d'articles dans les chapitres 3 et 4. En effet, le chapitre 3 s'intéresse à la détermination de deux extraits végétaux potentiels pour lutter contre les plantes indésirables, plus particulièrement *Trifolium incarnatum* (*T. incarnatum*), *Sylibum marianum* (*S. marianum*) et *Phalaris minor* (*P. minor*). Le premier extrait est l'huile essentielle de *Rosmarinus officinalis* et le deuxième est l'extrait

phénolique de *C. Cardunculus*. Après avoir sélectionné ces 2 extraits, l'étude des modes d'actions a été débuté par l'interaction de ces extraits avec la membrane plasmique de la plante (chapitre 4). Le chapitre 5 met e, evidence l'acitivté des molécules bioactives dont certains composés phénoliques originaux, ce qui a amené au dépôt brevet. Finalement, le chapitre 6 comprend une discussion générale, les conclusions et les perspectives de la thèse.

Données bibliographiques

1. Contexte et problématiques

Au cours des dernières décennies, l'explosion démographique a exercé une pression immense sur la production agricole, obligeant d'intensifier de manière plus durable l'agriculture afin de répondre à la demande alimentaire qui ne cesse d'augmenter (Bajwa et al., 2015). La production agricole doit augmenter de 70% pour nourrir une population mondiale qui devrait atteindre 9,6 milliards d'ici 2050 (van Evert et al., 2017). En parallèle, les pertes de récoltes dues aux mauvaises herbes continuent de réduire la production disponible dans le monde entier. Ces pertes de rendement (environ 32%) sont supérieures à celles causées par des parasites (18%) ou des agents pathogènes (15%) (van Evert et al., 2017). Pour cela la gestion des mauvaises herbes revêtira une importance cruciale afin d'assurer la sécurité alimentaire mondiale.

Dans les systèmes agricoles, il a été démontré que les mauvaises herbes sont des ravageurs des plantes qui peuvent réduire les rendements des cultures et nuire aux fonctions des plantes pour freiner leur croissance. Les mauvaises herbes ont été considérées comme des espèces envahissantes causant des changements dramatiques dans les systèmes écologiques et les champs agricoles, car elles altèrent profondément les communautés et les écosystèmes (Watanabe et al., 2014). Elles sont constamment en concurrence avec les cultures pour les ressources en eau et en éléments nutritifs, ce qui réduit les rendements et la qualité et entraîne par conséquent des pertes économiques énormes (Araniti et al., 2015).

D'autres part, les pratiques agricoles intensives, les changements climatiques et l'augmentation du nombre de plantes envahissantes rendent la situation plus complexe et laissent un grand débat politique sur les solutions possibles. Parmi ces solutions, l'utilisation des pesticides chimiques fait actuellement l'objet de discussions critiques entre différents acteurs et entreprises privées et la réduction des risques pour la santé humaine et l'environnement liés à l'utilisation de pesticides figure en tête des priorités des décideurs de l'industrie alimentaire (Böcker et al., 2019).

Il est donc vital pour l'agriculture de disposer d'alternatives durables afin d'assurer le bon déroulement des cultures et de garantir une alimentation saine aux consommateurs. Les méthodes alternatives (thermiques, mécaniques) proposées actuellement pour lutter contre les adventices se montrent peu efficaces dans le temps. Cependant, les agriculteurs ont recours à l'utilisation des herbicides traditionnels chimiques qui peuvent être nocifs pour l'environnement et la santé

humaine (Jabran et al., 2015).

Il a été démontré que l'utilisation intensive des herbicides de synthèse principalement le glyphosate induit d'une part le risque d'impacts néfastes sur l'environnement, l'animal et la santé humaine et, d'autre part, l'apparition d'adventices résistantes (211 espèces et 393 biotypes résistants identifiés, dont 25 espèces pour le glyphosate en 2015). En plus, Il n'existe quasi pas de bioherbicides commercialisés à part ceux à base d'acide pélargonique ou d'acide acétique (Soltys et al., 2013). Pour cette raison, l'industrie des herbicides devrait introduire des classes d'herbicides dotées de nouveaux mécanismes d'action afin de lutter contre l'évolution de la résistance des adventices aux herbicides.

2. Allélopathie et les différentes méthodes de contrôle des adventices

2.1 Les adventices

2.1.1 Définition

Par définition, une adventice est n'importe quelle plante qui pousse dans un endroit sans y avoir été intentionnellement installée (Soltys et al., 2013). Elle se retrouve évidemment dans les champs mais aussi en exploitation forestière (fougère par exemple), sur les infrastructures (bâtiments, voies ferrées, ...) et dans les parcs et jardins (Tesio & Ferrero, 2010). En effet, ces adventices rentrent en concurrence avec les plantes cultivées pour l'accès aux ressources (eau, nutriments et lumière). Celles-ci présentent plusieurs désavantages qui expliquent la mise en place de méthodes de lutte visant leur contrôle (Jabran et al., 2015).

2.1.2 Effet des adventices sur la production agricole

Les adventices représentent la première cause de diminution des rendements de la production agricole avec des réductions de rendement pouvant aller jusqu'à 32 % (van Evert et al., 2017). D'autre part, aux États-Unis, la prolifération d'espèces de plantes envahissantes provoque des dommages économiques estimés à 120 milliards de dollars (Rial et al., 2014). Parmi les espèces problématiques, on peut citer *Echinochloa crus-galli* et *Urochloa decumbens*. Elles sont très compétitives pour les plantes indigènes, et peuvent modifier de manière marquée l'environnement dans lequel elles dominent. Elles causent des pertes pouvant atteindre 70% dans les rizières et peuvent également toucher d'autres cultures telles que le coton, le maïs et la pomme de terre (Rial et al., 2014).

2.2 Allélopathie

2.2.1 Définition

D'après Macias et al., (2007), l'allélopathie est l'ensemble de plusieurs interactions biochimiques directes ou indirectes, positives ou négatives. Elle fait intervenir des substances allélochimiques qui interviennent dans la communication interspécifique.

2.2.2 Composés allélochimiques

L'effet allélopathique d'une espèce végétale sur une autre espèce peut être constitutif ou induit et dépend de la régulation de la synthèse et de la libération des allélochimiques qui sont influencées par des facteurs génétiques mais aussi par l'environnement, comme par exemple la température, les nutriments et l'irradiation et les facteurs biotiques. Peu de mécanismes de régulation de la production allélochimique sont actuellement connus et la reproductibilité des effets allélopathiques dans les conditions de terrain est faible (Chengxu et al 2011). La libération des substances allélochimiques, souvent des métabolites secondaires, se fait par des mécanismes variés : volatilisation à partir des feuilles, exsudation des racines et lixiviation des feuilles et de la litière sur le sol par les précipitations (Figure 1)(Chengxu et al 2011).

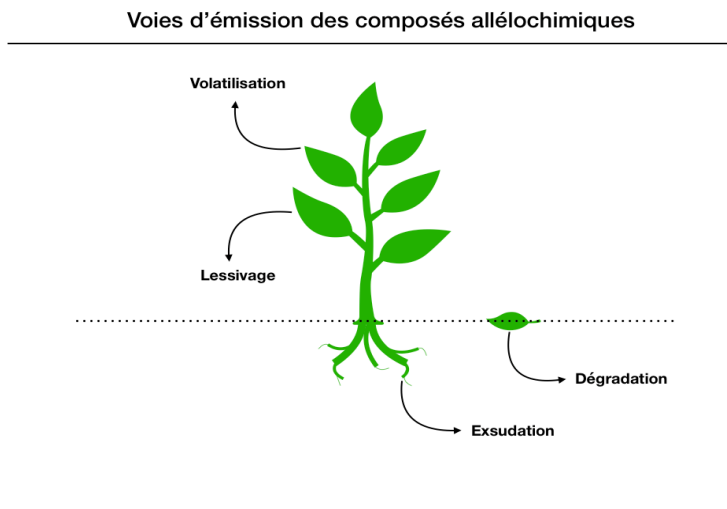


Figure 1 : Voies d'émission des composés allélochimiques

Ces substances ont un effet direct sur la germination et la croissance des plantes voisines en perturbant plusieurs processus physiologiques, notamment la photosynthèse, la respiration, l'équilibre hydrique et hormonal (Weir et al., 2004). Comme le montre la figure 2, la plupart des composés allélochimiques pénètrent dans le sol sous forme de composés déjà actifs pour les plantes, par exemple ; acides phénoliques, cyanamide, momi-lactones, héliannuols, etc. Certains doivent être modifiés en forme active par des micro-organismes ou par des conditions environnementales spécifiques (pH, humidité, température, lumière, oxygène, etc.), par exemple ; la juglone, la benzoxazoline-2-one (BOA), le 2-amino-3-H-phénoxazin-3-one (APO).

Transport des composés allélochimiques

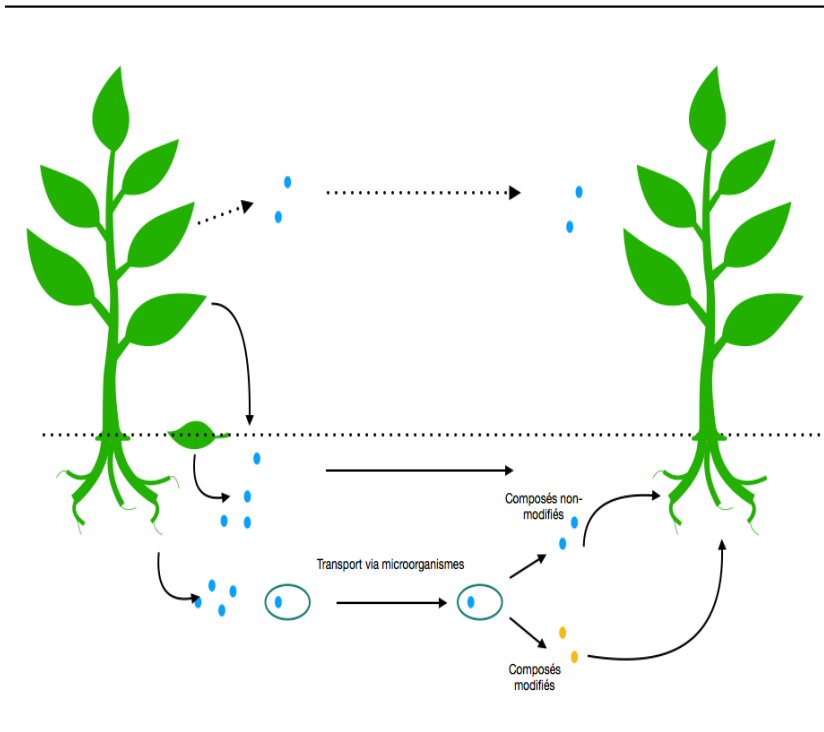


Figure 2: Transport des composés allélochimiques.

D'autres part, les composés allélochimiques sont classés en fonction de leurs similarités chimiques (figure 3) : (1) acides organiques solubles dans l'eau, alcool à

chaîne droite, aldéhydes aliphatiques et cétones ; (2) lactones simples insaturés ; (3) acides gras à chaînes longues et polyacéthylènes ; (4) quinines (benzoquinones, anthraquinones et quinines complexes); (5) phénols, acide benzoïque et ses dérivés ; (6) acide cinnamique et ses dérivés ; (7) coumarines ; (8) flavonoïdes ; (9) tannins ; (10) terpénoïdes (sesquiterpène lactones, diterpènes, et triterpénoïdes).

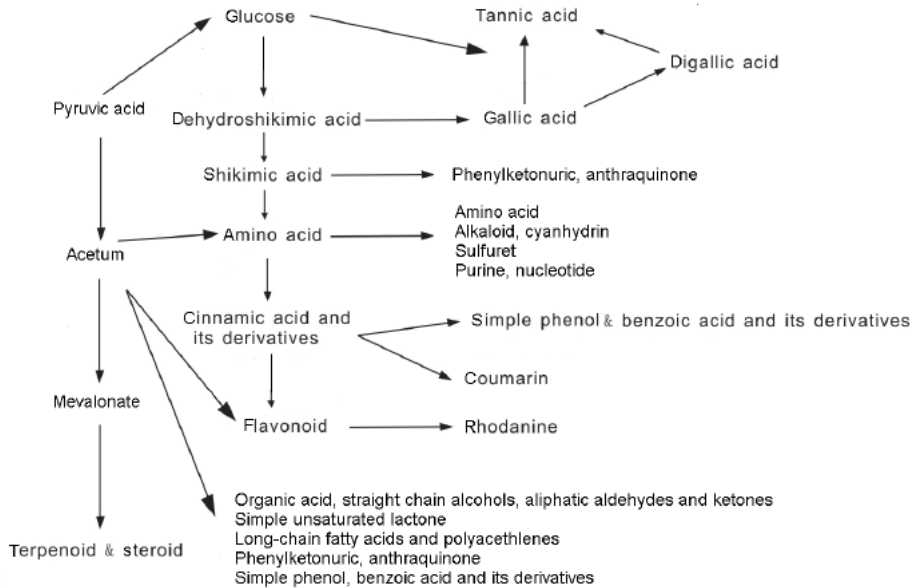


Figure 3: Grandes familles de composés allélochimiques et leurs voies de biosynthèse communes provenant de nombreux précurseurs clés.

2.2.3 Rôle de l'allélopathie dans la gestion des adventices

Jusqu'à présent l'allélopathie a été décrite en tant qu'un outil de gestion des mauvaises herbes et son potentiel a été négligé. Une compréhension plus approfondie de ce phénomène ouvrira la voie à une meilleure utilisation de l'allélopathie dans la production agricole. Plusieurs cultures telles que le tournesol (*Helianthus annuus*), le sorgho (*Sorghum bicolor*), le riz (*Oryza sativa*), le colza (*Brassica napus*), le blé (*Triticum aestivum*) et l'orge (*Hordeum vulgare*) sont connus pour leur potentiel allélopathique (Soltys et al., 2013 ; Jabran et al., 2015).

De nombreuses études confirment l'importance de l'utilisation des cultivars allélopathiques afin de réduire la pression exercée par les adventices. En effet, le tableau 1 donne quelques exemples de cultivars allélopathiques qui ont la capacité

d'éliminer les adventices à travers la production de composés allélochimiques (Jabran et al., 2015).

Tableau 1 : Quelques cultivars allélopathiques qui peuvent être efficaces dans la lutte contre les adventices

Culture	Cultivars	Adventices	Pays	Allélochimique	Efficacité (%)
Blé	Athinaida	<i>Echinochloa crus-galli</i> (L.)	Grèce	-	83
Riz	Niundari baekna	<i>Echinochloa crus-galli</i> (L.)	Corée	Momilactone A	>50
	Janganbyeon	<i>Echinochloa crus-galli</i> (L.)	Corée	<i>p</i> -hydroxybenzoic acid	79-94
	Dinorado	<i>Echinochloa crus-galli</i> (L.)	Iran	Acides phénoliques	60
	OM 5930	<i>Lepidium sativum</i> L	Etats unis-Vietnam	<i>N-trans</i> -cinnamoyltyramine	-
Tournesol	Sin-Altheeb	<i>Rumex dentatus</i> L	Irak	Composés phénoliques	74

Certaines plantes de couverture comme le cas du tournesol améliorent également la suppression des adventices et par conséquent diminuent l'utilisation d'herbicides synthétiques. La présence de résidus de plantes de couverture sert de barrière physique à l'émergence d'adventices (Jabran et al, 2015). De plus, elles libèrent des substances biochimiques efficaces qui peuvent affecter la germination des graines et la croissance d'adventices (Tesio & Ferrero, 2010).

En effet, dans la littérature, les composés allélochimiques issus des plantes qui montrent un effet allélopathique négatif peuvent être utilisés en tant que traitement pour moduler la croissance de certaines plantes néfastes (Vyvyan, 2002; Liu et al., 2017 ; Sbai et al 2016). En effet, ces composés allélochimiques peuvent avoir un potentiel intéressant en agriculture comme herbicides naturels. En plus, la diversité et la variabilité de ces composés sont des outils prometteurs possédant des propriétés spécifiques pour la découverte de nouveaux sites d'action pour des nouveaux produits. (Ben Kaab et al., 2019a). Même s'ils inhibent la photosynthèse ou la

respiration, ils peuvent également se lier à des protéines et à des sites différents de ceux des herbicides synthétiques. Ces substances allélochimiques sont également caractérisées par une action multisite chez les plantes sans une grande spécificité comme dans le cas des herbicides synthétiques (Soltys et al., 2013).

Plusieurs composés allélopathiques isolés à partir de plantes peuvent être utilisés comme bioherbicide. Parmi ces composés, on peut citer la sorgoleone, momilactone, artemisinin, leptospermon, les huiles essentielles d'eucalyptus et la sarmentine (Soltys et al., 2013)

2.3 Moyens de lutte contre les adventices

Afin de contrôler les adventices dans les cultures, plusieurs méthodes (figure 4) peuvent être utilisées et selon HRAC, la gestion intégrée des mauvaises herbes est définie comme l'utilisation d'une gamme de techniques de contrôle, englobant les méthodes physiques, chimiques et biologiques de manière intégrée sans recours excessif à une seule méthode. Ces techniques, lorsqu'elles sont utilisées dans une approche rotative et intégrée, aideront à réduire la pression de sélection sur toutes les espèces de mauvaises herbes limitant ainsi considérablement les chances de survie des mauvaises herbes résistantes.

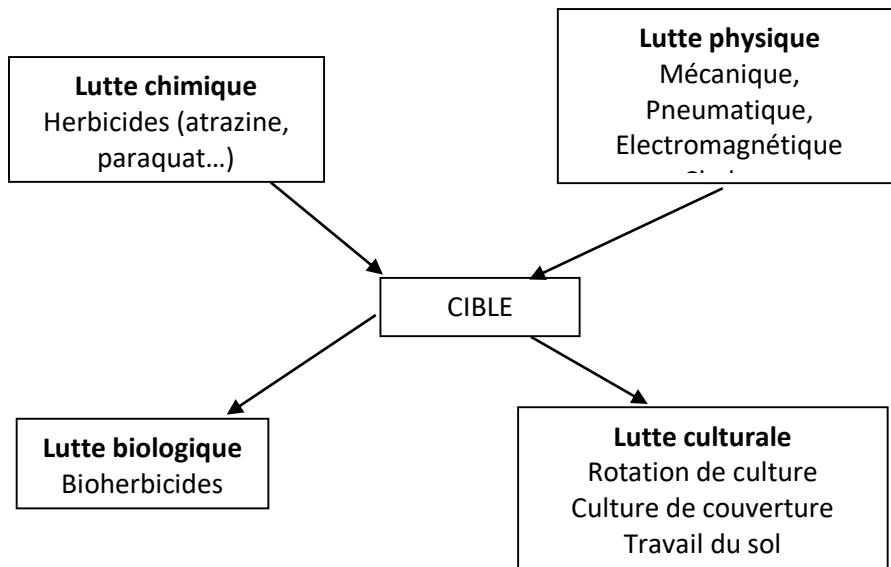


Figure 4 : Les différents moyens de lutte contre les adventices

2.3.1 Lutte Culturelle

Plusieurs pratiques agricoles sont mises en évidence pour éviter l'émergence des mauvaises herbes dans les champs des cultures. Parmi ces méthodes, Bajwa et

al., (2015) ont cité :

- Le travail du sol (Travail en bande pour cibler les mauvaises herbes)
- La gestion des éléments nutritifs des cultures
- Le contrôle des semences de cultures.

Par ailleurs, la rotation des cultures est considérée comme une des méthodes culturales pour diminuer l'emploi des herbicides. Certaines cultures montrent des avantages compétitifs, réduisant ainsi l'impact des adventices associées avec elles (Jabran et al., 2015).

-Le faux semis qui consiste à créer des conditions favorables à la germination des graines d'adventices afin de les détruire avant le semis de la bonne culture.

2.3.2 Lutte physique

Selon Melander et al., (2005), plusieurs méthodes sont disponibles en lutte physique :

- Le retournement de la couche supérieure de la terre pour enfouir les plantes indésirables
- Le désherbage manuel et l'arrachage. Il peut se faire soit directement à la main ou à l'aide d'appareils qui arrachent mécaniquement les adventices
- La méthode thermique qui utilise les hautes températures pour détruire les adventices.

Parmi toutes ces méthodes, certaines peuvent se montrer plus efficaces que d'autres et cela en fonction de l'état de la culture, du sol et des moyens économiques dont dispose l'agriculteur. Elles ont tout de même l'avantage d'être plus respectueuses de l'environnement en limitant la pollution des nappes, des eaux de surface et se montrent moins nocives pour la santé du producteur bien que certaines précautions restent à prendre (Melander et al., 2005). Par contre dans certains cas, ces pratiques ne sont pas toujours les plus efficaces pour enrayer les populations d'adventices. Certaines espèces d'adventices peuvent présenter des systèmes racinaires plus importants qui sont souvent difficiles à enrayer (ex : chiendent, chardon des champs qui présente des rhizomes). Afin de contrôler plus facilement les populations d'adventices et avoir une action qui dure plus longtemps, le recours à l'utilisation de produits phytopharmaceutiques est devenu indispensable (Böcker et al., 2019).

2.3.3 Lutte chimique

2.3.3.1 Généralités sur les herbicides

Au cours de ces dernières décennies, les moyens de lutte utilisés contre les adventices sont dominés par l'utilisation des herbicides chimiques. En effet, ces derniers, présentent un coût faible et une efficacité importante (Davis & Frisvold, 2017). Par

contre, certains produits persistent longtemps dans l'environnement, contaminent les eaux et perturbent la faune et la flore (glyphosate, acétonifène, métobromuron). Ces produits chimiques présentent des risques sur la santé humaine et certains sont même classés cancérigènes probables (Cordeau et al., 2016). D'ailleurs, l'utilisation de nombreux produits phytosanitaires, au fil des années, a été interdite du fait de leur toxicité élevée et des conséquences engendrées par leurs utilisations (Heap, 2014). Dans ce contexte, une utilisation de ces produits à des stades inappropriés et lors de conditions climatiques défavorables peut conduire à une accumulation importante des matières actives dans les sols et entraîner par la suite une résistance chez les plantes cibles (Davis & Frisvold, 2017 ; Heap, 2014). Ces produits sont classés selon leur mode de pénétration :

- Les herbicides foliaires de contact qui agissent localement sur les parties aériennes de la plante
- Les herbicides foliaires systémiques qui agissent sur l'ensemble de la plante car la matière active du produit circule dans le système vasculaire
- Les herbicides racinaires systémiques qui circulent dans le système racinaire (Fredon Lorraine, 2013).

Néanmoins, l'évolution de l'utilisation des herbicides chimiques semble être nécessaire. Cette évolution vers une lutte dite intégrée s'explique du fait de la grande préoccupation à la fois des autorités et de la population vis-à-vis des dégâts qui ont déjà été engendrés par une utilisation abusive des pesticides. Cet excès a notamment soulevé les inquiétudes concernant les effets secondaires des pesticides. Ces effets secondaires sont nombreux : effets sur l'environnement avec la pollution des eaux, du sol et de l'air, effets sur la faune et la santé des agriculteurs et des consommateurs (perturbations endocriniennes, cancers...) (Pavela & Benelli, 2016).

2.3.3.2 Mode de pénétration

Le mode de pénétration renseigne sur la façon dont l'herbicide entre dans la plante à travers l'organe qui sera ainsi touché. La pénétration peut se faire via les tissus méristématiques (cotylédons, gemmules, bourgeons), les racines (herbicide racinaire) ou les feuilles (herbicide foliaire). Si l'herbicide pénètre superficiellement à la surface de l'organe touché et s'il n'agit qu'à cet endroit, on parle d'herbicide de contact. Tandis que s'il pénètre dans la plante et y circule on parle d'herbicide systémique (Creech et al., 2018).

Les herbicides de contact détruisent essentiellement en nécrosant les parties atteintes lors de l'application du produit. Ils ne se déplacent que faiblement dans la

plante et leur action est généralement rapide. Par contre, les herbicides systémiques sont eux absorbés par la partie de la plante sur laquelle ils sont appliqués ; ils sont ensuite véhiculés dans la plante et peuvent ainsi exercer une action phytotoxique dans d'autres parties de la plante. La pénétration et la migration dans la plante peut se faire par les stomates, les trichomes ou à travers la cuticule en elle-même. Cette action est en général plus lente que celle des herbicides de contact (Creech et al., 2018 ; Wang & Liu, 2007).

2.3.3.3 Mode d'action des herbicides

La plupart des herbicides commerciaux ciblent des enzymes impliquées dans des mécanismes importants chez la plante (tableau 2) (Dayan et al, 2012). Les sites d'action ciblés par ces produits sont la photosynthèse, la synthèse de pigment photosynthétique et des acides aminés, le transfert d'énergie, l'expression et la régulation de gènes, la régulation hormonale, la macrostructure (polymérisation des microtubules, synthèse de cellulose), la synthèse de lipides, la fonction des membranes et la stabilité des lipides, le cycle cellulaire de la plante. Par exemple, l'atrazine est un inhibiteur du photosystème II (PSII), le chlorsulfuron inhibe la synthèse d'acétolactate synthase, le dicamba inhibe la synthèse d'auxines et le glyphosate cible le 5-énoypyruvylshikimate-3 phosphate synthase (EPSPS) (Shariq I. Sherwani, 2016).

Tableau 2 : Sites d'action de quelques herbicides chimiques (Shariq I. Sherwani, 2016 ; Varanasi et al., 2015)

#	Herbicide group	Group	Example of Herbicide	Dicots	Monocots	Total
1	ALS inhibitors	B	Chlorsulfuron	101	64	165
2	Photosystem II inhibitors	C1	Atrazine	51	23	74
3	ACCase inhibitors	A	Sethoxydim	0	49	49
4	EPSP synthase inhibitors	G	Glyphosate	24	24	48
5	Synthetic Auxins	O	2,4-D	33	8	41
6	PSI Electron Diverter	D	Paraquat	22	10	32
7	PSII inhibitor (Ureas and amides)	C2	Chlorotoluron	11	18	29
8	PPO inhibitors	E	Oxyfluorfen	10	3	13
9	Microtubule inhibitors	K1	Trifluralin	2	10	12
10	Lipid Inhibitors	N	Triallate	0	10	10
11	Long chain fatty acid inhibitors	K3	Butachlor	2	5	7
12	Carotenoid biosynthesis (unknown target)	F3	Amitrole	1	5	6
13	PSII inhibitors (Nitriles)	C3	Bromoxynil	3	1	4
14	Carotenoid biosynthesis inhibitors	F1	Diflufenican	3	1	4
15	Glutamine synthase inhibitors	H	Glufosinate-ammonium	0	4	4
16	Cellulose inhibitors	L	Dichlobenil	0	3	3
17	Antimicrotubule mitotic disrupter	Z	Flamprop-methyl	0	3	3
18	HPPD inhibitors	F2	Isoxaflutole	2	0	2
19	DOXP inhibitors	F4	Clomazone	0	2	2
20	Mitosis inhibitors	K2	Propham	0	1	1
21	Unknown	Z	Endothall	0	1	1
22	Cell elongation inhibitors	Z	Difenzoquat	0	1	1
23	Nucleic acid inhibitors	Z	MSMA	1	0	1

2.3.3.4 Résistance aux herbicides

Selon HRAC, la résistance aux mauvaises herbes est la capacité héréditaire naturelle de certains biotypes de mauvaises herbes de survivre à un traitement herbicide. Dans le système de classification HRAC, il existe 25 groupes d'herbicides (figure 5).

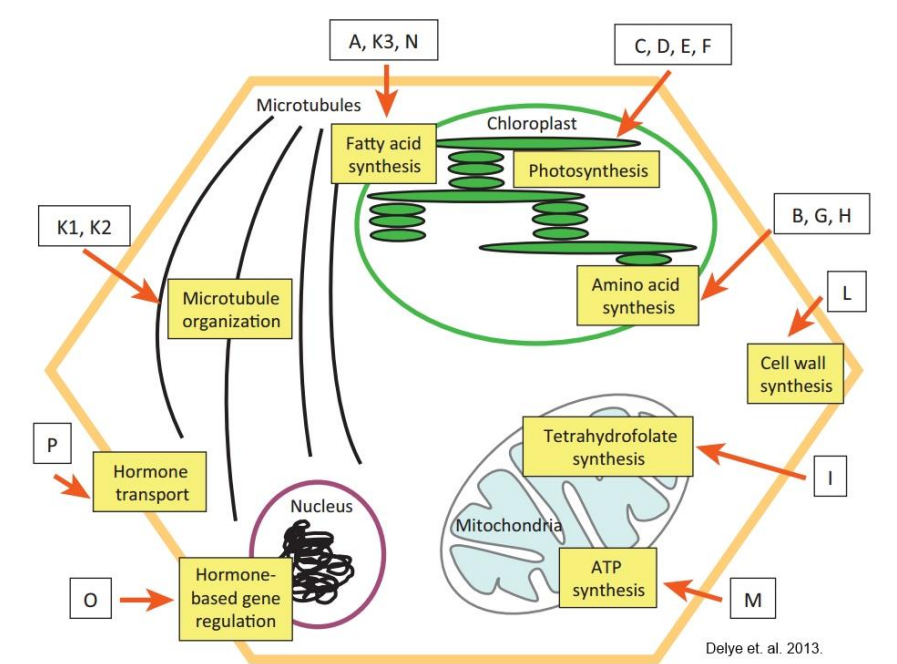


Figure 5: Les sites d'action de quelques herbicides chimiques selon le comité de la résistance aux herbicides (HRAC).

Selon Soltys et al., (2013), 211 espèces et 393 biotypes d'adventices ont été identifiés comme résistants aux herbicides. 404 espèces (espèces × site d'action) de mauvaises herbes sont résistantes aux herbicides à l'échelle mondiale (Figure 6). Les mauvaises herbes résistantes aux inhibiteurs de ALS représentent environ le tiers de tous les cas (133/404) et sont particulièrement gênantes pour le riz et les autres céréales. Contrairement aux herbicides chimiques qui présentent toujours une spécificité par rapport à un site d'action bien déterminé, les bioherbicides à base de molécules allélochimiques se distinguent davantage en vue de leurs actions multi-sites (Radhakrishnan et al., 2018). Des mutations dans des gènes cibles confèrent des résistances aux herbicides (Varanasi et al., 2015).

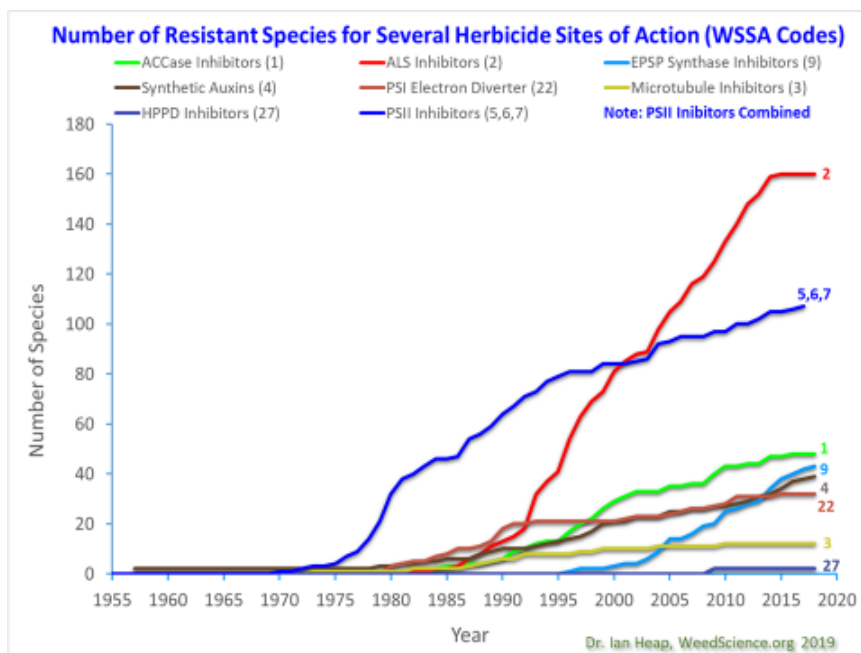


Figure 6 : Nombre d'espèces montrant une résistance pour les principales familles de molécules actives des herbicides chimiques (International Survey of Herbicide Resistant Weeds).

2.3.4 Bioherbicides (BH)

2.3.4.1 Définition

Selon Bailey, (2014), les bio-herbicides sont des produits d'origine naturelle permettant de lutter contre les mauvaises herbes. Ils peuvent être soit des organismes vivants, et plus particulièrement des micro-organismes, ou des produits dérivés d'organismes vivants, y compris les métabolites naturels produits par ces organismes au cours de leur croissance et de leur développement.

2.3.4.2 Généralités

Étant donné l'impact négatif des herbicides chimiques sur la santé humaine et l'environnement, les bioherbicides, composés de substances naturelles, représentent une alternative pertinente pour les agriculteurs. Leur efficacité à court terme est souvent moindre que les produits de synthèse (Cordeau et al., 2016). Cependant, leurs rémanences dans l'environnement et leurs écotoxicités sont souvent bien moins importantes. D'autre part, en utilisant ces substances actives naturelles, la

chaîne trophique écologique sera bien équilibrée et la biodiversité bien conservée (Duke et al., 2000 ; Dayan et al., 2009 ; Bailey, 2014).

Les bio-insecticides représentent pour l'instant la plus grande partie des produits de biocontrôle (46%), viennent ensuite les biofongicides (44%) et en dernière position les bioherbicides (1%) (Figure 7).

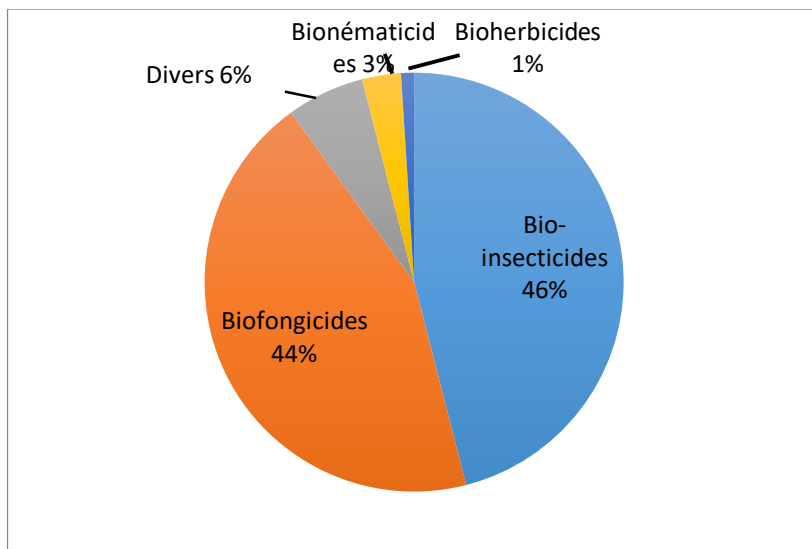


Figure 7 : Répartition du marché mondial des produits de biocontrôle « Union des Industries de la Protection des Plantes (UIPP) ».

2.3.4.3 Produits et substances actives d'origine naturelle sur le marché en 2019

Malgré les nombreux efforts au cours de ces dernières décennies pour développer des formulations BH, seule une dizaine de produits existent actuellement sur le marché au niveau mondial (Tableau 3). La plupart d'entre-eux utilisent des champignons ou des bactéries comme agents herbicides (Cordeau et al., 2016). En Europe, seules deux molécules actives produites à partir des plantes ont été commercialisées : l'acide pélargonique et l'acide acétique.

Tableau 3 : Quelques bioherbicides sur le marché mondial (Bailey, 2013)

Agent de biocontrôle ou substance active	Produit commercial	Usage	Pays où le produit est autorisé
<i>Colletotrichum gloeosporioides f. sp. aeschynomene</i> souche ATCC 20358	LockDown ® (anciennement Collego ®) ¹	Contrôle d' <i>Aeschynomene virginica</i> (L.)	USA
<i>Puccinia thlaspeos</i> C. Shub.	Woad Warrior ®	Contrôle de <i>Isatis tinctoria</i> L.	USA
<i>Chondostereum purpureum</i> (Fr.) Pouz.	Chontrol ®	Contrôle des tiges de <i>Prunus serotina</i> Ehrh. et de <i>Populus euramericana</i> Guinier.	USA et Canada
<i>Alternaria destruens</i> L. Simmons. souche 059	Smoulder ®	Contrôle des plantes du genre <i>Cuscuta</i>	USA
<i>Sclerotinia minor</i> Jagger souche IMI 344141	Sarritor ®	Contrôle des dicotylédones présentes en pelouse.	Canada
Fer chélaté sous forme de FeHEDTA (acide hydroxyéthylènediamine triacétique)	EcoSense ® Weed B Gon ® Fiesta ® Iron X ®, NEU1173 ®	Terrains non agricoles (pelouses, parcs, terrains sportifs, sentiers, ...)	Canada, USA (dépend du produit)
<i>Phoma macrostoma</i> souche 94-44B	Phoma	Contrôle des dicotylédones présentes sur terrains de golf	USA et Canada
Thaxtomine A, substance produite par fermentation de la bactérie <i>Streptomyces acidiscabies</i> souche RL-110.	Opportune ® , MBI ®	Contrôle de <i>Taraxacum officinale</i> F. H. Wigg présent en pelouse, prairies ou sur terrains de golf.	Opportune ® au Canada et MBI ® aux USA.
<i>Xanthomonas campestris</i> souche JTP482	Camperico ®	Contrôle de <i>Poa annua</i> L. en pelouse.	Japon
Mélange de différentes bactéries du genre <i>Lactobacillus</i> produisant de l'acide lactique et de l'acide citrique	Kona ® ou Bioprotec Herbicide ® (anciennement Organo-Sol ®)	Contrôle de fabaceae en pelouse et prairie.	Canada
Acide pélargonique (ou acide nonanoïque)	Beloukha®	Contrôle des adventices en vignes ou défanant pour culture de pommes de terre. Probablement	Union Européenne ⁵ . Probablement bientôt autorisé

		autorisé bientôt en arboriculture et pour les jardins.	aux USA et au Japon ¹ .
	Katoun ® ^{1, 3} , Compo ® anti-mousse gazon et Compo ® désherbant & anti-mousse ³	Contrôle des adventices en terrains non agricoles (parcs, jardins, terrains sportifs et sentiers).	Union Européenne
	Finalsan ® ⁴	Contrôle des adventices en pépinières, cultures ornementales et terrains non agricoles	Union Européenne
Acide acétique	Cito Global ®, Naturen ®	Contrôle des adventices dans toutes les cultures et en terrains non agricoles (parcs, jardins, terrains sportifs et sentiers).	Union Européenne.

2.3.4.4 Produits et substances actives synthétiques analogues de molécules naturelles

Plusieurs substances herbicides de synthèse ont été développées sur le modèle d'analogues naturels : c'est le cas, par exemple, de la leptospermone utilisée pour développer la famille des tricétones (Sulcotrione® et Mesotrione®) qui fait partie des inhibiteurs de la 4-hydroxyphénylpyruvate dioxygénase (Cordeau et al., 2016 ; Duke et al., 2000). Des molécules naturelles aux propriétés herbicides ont été découvertes : le bialaphos isolé de *Streptomyces* spp. (agit biologiquement grâce à la phosphinothricine, version biosynthétisée du glufosinate) (Duke et al., 2000 ; Dayan et al., 2009). Une molécule analogue du 1,4-cinéole, la cimméthylène a également été développée pour présenter des propriétés permettant la production d'un herbicide (Duke et al., 2000 ; Duke et al., 2003).

3 Les extraits végétaux et leurs potentiels herbicides

3.1 Métabolites secondaires : les composés phénoliques et les huiles essentielles

3.1.1 Généralités

Les métabolites primaires, tels que des phytostérols, des lipides d'acyle, des nucléotides, des acides aminés et des acides organiques, sont des composés qui ont des rôles essentiels associés à la photosynthèse, la respiration, la nutrition et les processus métaboliques essentiels à l'intérieur de la plante tandis que les métabolites secondaires (MS) ont un rôle

essentiel dans les interactions écologiques entre la plante et son environnement (Croteau et al., 2000). Ces métabolites diffèrent considérablement d'une espèce à l'autre. Cela peut dépendre de l'origine des plantes, du climat, de la qualité du sol. Dans ce contexte, de nombreuses études ont montré que certains de ces composés participent à des interactions allélopathiques (Soltys et al., 2013). Dans le cadre de ce manuscrit, Une attention particulière sera accordée aux composés phénoliques et les huiles essentielles qui ont été évoqués dans cette étude.

3.1.2 Composés phénoliques

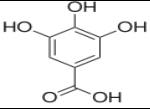
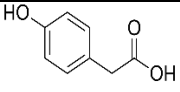
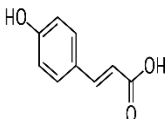
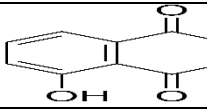
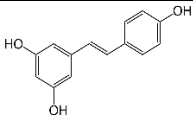
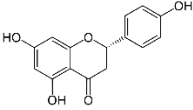
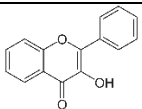
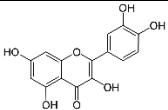
(i) définition et généralités

Parmi les métabolites secondaires des plantes, les polyphénols représentent un groupe important et diversifié de substances présentes en abondance dans la majorité des fruits, des herbes et des légumes. Les polyphénols représentent un groupe important d'au moins 10 000 composés différents contenant un ou plusieurs cycles aromatiques auxquels un ou plusieurs groupes hydroxyle sont liés (Falleh et al., 2008). Le terme polyphénols désigne toute substance chimique possédant au moins dans sa structure un noyau aromatique, attaché à un ou plusieurs groupements hydroxyles. Ils constituent une famille de molécules très largement répandues dans le règne végétal (Crosier et al., 2006). Ils sont responsables de la couleur des fruits rouges, des jus de fruits et des vins et des substrats pour le brunissement enzymatique, ainsi que des propriétés gustatives (Ve et al, 2012). Ces composés jouent un rôle important dans les mécanismes de défense des plantes contre les stress biotiques et abiotiques. Elles pourraient constituer une partie importante du système de défense des plantes contre les parasites et les maladies, notamment les nématodes parasites des racines. Ces molécules sont largement répandues dans les feuilles, les tiges, les graines et les fruits de nombreuses plantes comestibles (Falleh et al., 2011). Ils constituent l'un des groupes de substances antioxydantes les plus nombreux et les plus largement répandus dans le règne végétal, avec plus de 8 000 structures phénoliques connues à ce jour (Trabelsi et al., 2013).

(ii) Structure

Le tableau 4 représente les principaux composés phénoliques (Crosier et al., 2006).

Tableau 4 : Principaux composés phénoliques.

N°de carbone	Squelette	Classe	Exemple	Structure chimique
7	C ₆ -C ₁	Acide phénoliques	Acide gallique	
8	C ₆ -C ₂	Acides phénylacétique	Acide p-hydroxyphénylacétiques	
9	C ₆ -C ₃	Acides hydrocinnamiques	Acide p-coumariques	
10	C ₆ -C ₄	Naphtoquinones	Juglone	
14	C ₆ -C ₂ -C ₆	Stilbènes	Resvératrol	
15	C ₆ -C ₃ -C ₆	Flavonoïdes	Naringénine	
15	C ₆ -C ₃ -C ₆	Flavonoïdes	Myricitrine	
15	C ₆ -C ₃ -C ₆	Flavonoïdes	Quercétine	

(iv) Voie de biosynthèse

Les acides phénoliques sont synthétisés à partir de la voie shikimate de la L-phénylalanine ou de la L-tyrosine (Figure 8). La phénylalanine et la tyrosine sont des acides aminés très importants dans cette voie, car ces acides aminés sont les précurseurs courants de la majorité des produits phénoliques naturels. La désamination, l'hydroxylation et la méthylation sont les trois principales réactions impliquées dans la formation des acides phénoliques (Ewané et al., 2012). Ils présentent une grande diversité de structures, y compris des molécules assez simples

(par exemple, la vanilline, l'acide gallique, l'acide caféique) et des polyphénols tels que les stilbènes, les flavonoïdes et les polymères dérivés de ces différents groupes (Ve, 2012).

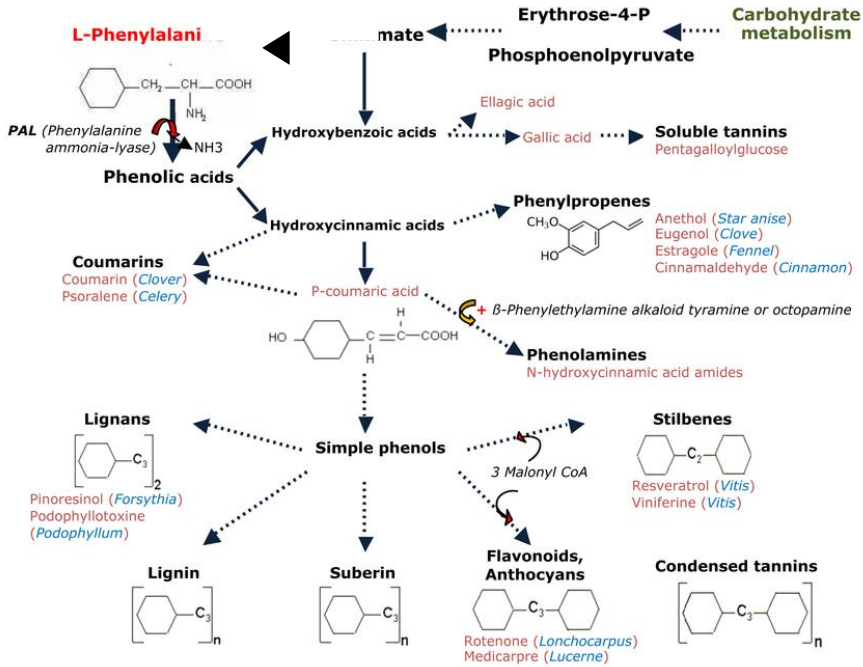


Figure 8 : Voie de synthèse simplifiée des composés phénoliques (adapté de (Ewané et al., 2012).

(v) Intérêts et utilisation des composés phénoliques

Les composés phénoliques présentent également de grandes propriétés physiologiques, telles que des effets anti-allergéniques, anti-arthérogènes, anti-inflammatoires, antimicrobiens, anti-thrombotiques, cardioprotecteurs et vasodilatateurs (Ksouri et al., 2007).

Les acides phénoliques sont largement étudiés et leur rôle dans la prévention des maladies neurodégénératives est prouvé. Certaines espèces végétales produisent des composés phénoliques pour inhiber la croissance d'autres plantes concurrentes. Dans ce contexte, ces composés jouent le rôle d'allélochimiques (Heleno et al., 2015). Ksouri et al., (2012) ont prouvé que ces composés phénoliques jouent un rôle important dans la neutralisation des radicaux libres et de l'oxygène singulet désactivé, ou des peroxydes en décomposition, qui sont inévitablement produits lorsque le métabolisme aérobie ou photosynthétique est altéré. Leur présence à l'intérieur de la cellule peut empêcher de nombreuses maladies.

(vi) Méthodes d'extractions

- **Méthodes conventionnelles**

Pendant de nombreuses années, les techniques conventionnelles ont été largement acceptées, principalement en raison de leur facilité d'utilisation, de leur efficacité et de leur large applicabilité (Figure 9). De tels procédés impliquent l'utilisation de solvants classiques tels que les alcools (méthanol, éthanol), l'acétone, l'éther diéthylique et l'acétate d'éthyle, souvent mélangés avec des proportions différentes d'eau. Par contre, l'utilisation de ces solvants présente plusieurs inconvénients : outre un éventuel effet dangereux sur la santé humaine, les résidus de solvants peuvent également rester dans les produits finis. Cela nécessite des étapes de purification supplémentaires qui prennent du temps et influencent le coût total du processus. De plus, en utilisant des solvants organiques purs, les acides phénoliques très polaires (acides benzoïque, cinnamique) ne peuvent pas être extraits complètement. Dans de tels cas, des mélanges alcool-eau ou acétone-eau sont suggérés. Les cires, les huiles, les stérols et la chlorophylle sont des composés hautement apolaires et peuvent être extraits du matériau par des solvants moins polaires tels que le dichlorométhane, le chloroforme, l'hexane et le benzène. (Arceusz et al., 2013; Khoddami et al., 2013)

- **Méthodes modernes**

En raison de problèmes liés aux températures de traitement élevées et aux temps de traitement longs dans les procédures d'extraction conventionnelles, il existe un besoin essentiel de promouvoir le développement et l'application de techniques d'extraction alternatives pour les composés phénoliques. Les alternatives possibles sont l'extraction assistée par ultrasons, l'extraction assistée par micro-ondes, l'extraction assistée par ultrasons-micro-ondes, l'extraction par fluide supercritique et l'extraction sous-critique par eau, qui ont récemment suscité un grand intérêt (Khoddami et al., 2013). En raison de leur simplicité, des temps d'extraction plus courts et de la consommation réduite de solvant organique. (Ajila et al., 2011).

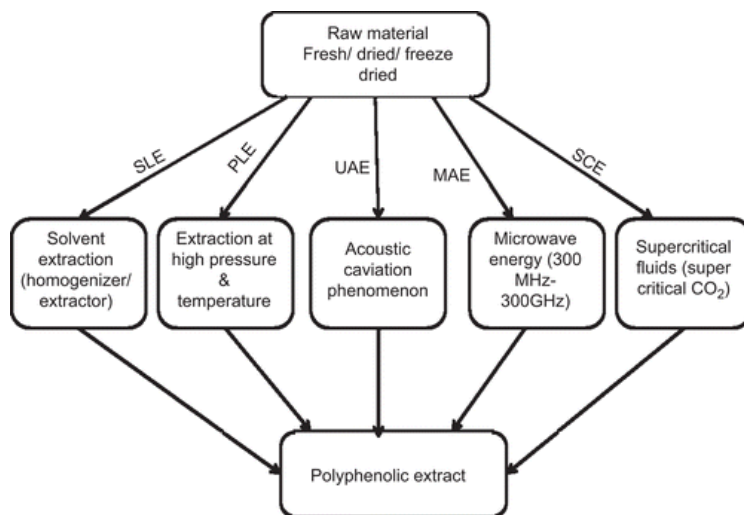


Figure 9 : Représentation schématique des différentes méthodes d'extraction: extraction solide-liquide , extraction liquide sous pression,extraction assistée par ultrasons, extraction assistée par micro-ondes et extraction super critique (SCE).

3.1.3. Les huiles essentielles (HE)

(i) Définitions et généralités

Les huiles essentielles sont des composés volatils, naturels et complexes (allant de 20 à 60 molécules différentes), caractérisés par une forte odeur. Elles sont formées par les plantes aromatiques comme métabolites secondaires. Elles sont obtenues à partir de parties de plantes par distillation à la vapeur. Ils contiennent un mélange de composés volatils (Koul et al., 2008).

Les huiles essentielles peuvent être classées en deux groupes basés sur l'origine biosynthétique de la ou des molécules dominantes représentant de 20 à 70% de l'extrait : Les HE terpéniques (terpénoides) et les HE aromatiques et aliphatiques (Bakkali et al., 2008).

Les terpénoïdes constituent la catégorie la plus variée de produits naturels à base de plantes. Ils constituent la plus grande classe de produits naturels avec plus de 55 000 composés connus structurellement diversifiés (Heleno et al., 2015). Généralement ils sont produits en grande quantité dans des pays tempérés à chauds comme les pays méditerranéens et tropicaux où ils représentent une partie importante de la pharmacopée traditionnelle (Bakkali et al., 2008). Les HE sont produits dans 17 500 espèces aromatiques de plantes supérieures appartenant principalement à quelques

familles, dont les Apiaceae, les Myrtaceae, les Lauraceae, les Lamiaceae et les Asteraceae. Cependant, seule une petite proportion (environ 300 espèces) a été utilisée dans des applications commerciales (Soltys 2013).

(ii) Structure

La figure 10 présente quelques exemples de structure de composés terpéniques.

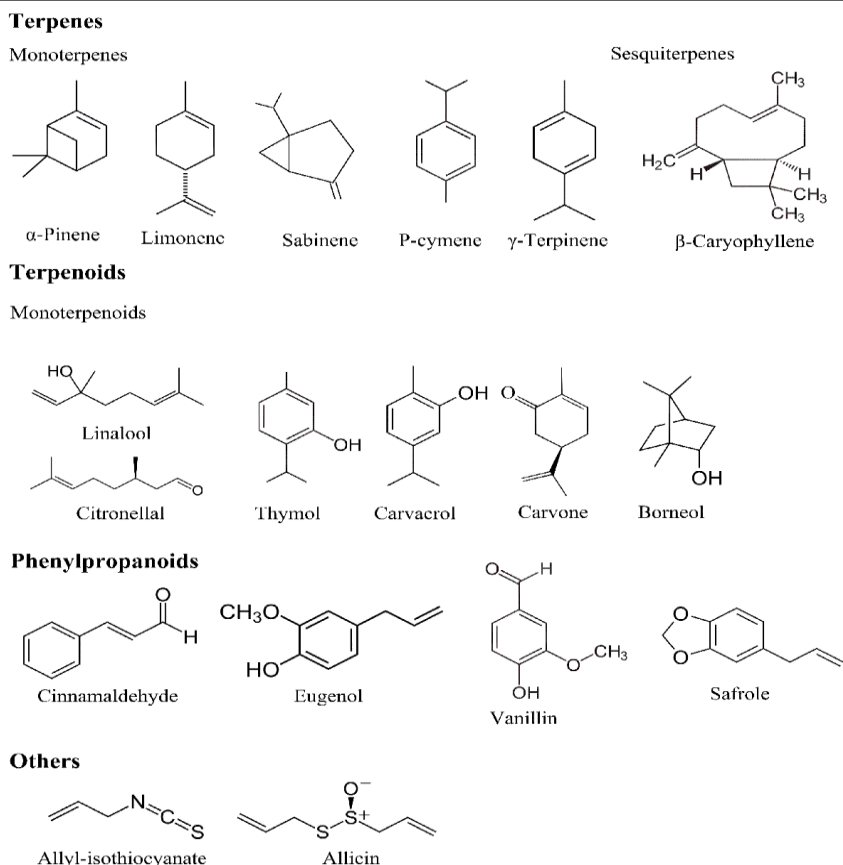


Figure 10 : Structures chimiques de certains constituants des huiles essentielles (Hyltdgaard et., 2012).

(iii) Voie de biosynthèse

Ces HE font partie du métabolisme secondaire des végétaux et proviennent d'unités isoprène C5 issues de deux voies de biosynthèse, par l'intermédiaire de l'acide mévalonique ou du 1-désoxy-D-xylulose 5-phosphate. Ces HE sont riche en composés terpéniques principalement des Monoterpenes (C10) des Sesquiterpènes

(C15), des Diterpènes (C20), des Triterpènes (C30,) et des Polyterpènes (C50) (Rehman et al., 2016).

(iv) Intérêts et utilisations des huiles essentielles

La grande majorité des différentes structures de terpènes produites par les plantes en tant que métabolites secondaires est impliquée dans la défense en tant que toxines et pour l'alimentation d'un grand nombre d'insectes et de mammifères. (Tohidi et al., 2019). En plus, Il a été démontré que les composés responsables de l'odeur sont des substances actives dotées de propriétés antibactériennes, fongicides et insecticides (Koul et al., 2008).

(v) Méthodes d'extractions

Les HE sont obtenues à partir de matières premières végétales par plusieurs méthodes d'extraction (Wang et Weller, 2006) (Dick et Starmans, 1996). Ces méthodes pourraient être classées en deux catégories : méthodes conventionnelles / méthodes avancées ou innovantes (Wang et Weller, 2006) (Dick et Starmans, 1996) . L'investigation dans les nouvelles technologies (ultrasons, micro-ondes) au cours des dernières décennies a conduit à l'émergence de nouveaux procédés d'extraction innovants et plus efficaces (réduction du temps d'extraction et de la consommation d'énergie, augmentation du rendement d'extraction, amélioration de la qualité des HE). Les méthodes conventionnelles et classiques sont des techniques basées sur la distillation de l'eau par chauffage pour récupérer les HE de la matrice de la plante. (Asbahani et al., 2015)

• Hydrodistillation

Cette méthode est la plus simple et la plus ancienne utilisée et le principe est basé sur la distillation azéotropique. En fait, à la pression atmosphérique et pendant le processus d'extraction (chauffage), les molécules d'eau et d'OE forment un mélange hétérogène qui a atteint sa température d'ébullition à un point inférieur proche de 100 ° C, ce qui est très élevé pour les composants d'OE (Reyes-Jurado et al., 2015)

- **Entraînement par la vapeur d'eau**
- C'est une méthode largement utilisée pour l'extraction des HE. Il est basé sur le même principe que l'hydrodistillation, à la différence qu'il n'y a pas de contact direct entre la plante et l'eau.
- **Extraction par solvant organique** Le matériel végétal est macéré dans un solvant organique; l'extrait est concentré en éliminant le solvant sous pression réduite. Cette technique évite les altérations et les artefacts chimiques par

extraction à froid par rapport à l'hydrodistillation. En effet, lors de l'hydrodistillation, l'immersion de la matière végétale dans l'eau du bowling provoque la solubilisation dans l'eau de certains constituants de parfum et réduit le pH moyen à 4–7 (parfois moins de 4 pour certains fruits). Les composants des espèces végétales d'origine sont soumis aux effets combinés de la chaleur et des acides, et sont sujets à des modifications chimiques (hydrolyse, déprotonations, hydratations et cyclisations). (Asbahani et al 2015)

- **Techniques innovantes d'extraction des huiles essentielles**

L'un des inconvénients des techniques conventionnelles est lié à la thermolabilité des composants de l'OE soumis à des altérations chimiques (hydrolyse, isomérisation, oxydation) en raison des températures élevées d'application donc extrêmement endommagé, particulièrement si le temps d'extraction est long. Il est important que les méthodes d'extraction permettent de maintenir la composition chimique et les proportions naturelles des HE dans leur état d'origine. Les nouvelles techniques d'extraction doivent également réduire les temps d'extraction, la consommation d'énergie, l'utilisation de solvants, l'utilisation de solvants et les émissions de CO₂. Ces Parmi ces techniques on peut citer l'extraction assistée par micro-ondes, l'extraction assistée par ultrasons et l'Extraction de fluide supercritique (SFE). (Reyes-Jurado et al., 2015)

3.2 Les plantes médicinales et aromatiques (PAM)

3.2.1 Généralités

Les PAM ont été employées pendant des siècles comme remèdes pour les maladies humaines parce qu'elles contiennent des composés de valeur thérapeutique. Les substances naturelles issues de ces plantes ont des intérêts multiples mis à profit dans l'industrie : en alimentation, en cosmétologie et en pharmacie. (Nostro et al., 2000). Cependant, peu d'études ont rapporté leurs utilisations comme agents herbicides en agriculture.

3.2.2 Présentation des espèces étudiées

(i) Présentation de *Rosmarinus officinalis*

- **Description botanique**

Le Romarin ou *R. officinalis* (figure 11) est un arbrisseau de la famille des Lamiacées (ou labiées), poussant à l'état sauvage sur le pourtour méditerranéen, en particulier dans les zones arides et rocailleuses et sur les terrains calcaires.



Figure 11 : *R. officinalis* collectée au stade de la floraison dans une montagne naturellement diversifiée de la région de Seliana, au nord-est de la Tunisie.

- **Intérêts et utilisations**

Le romarin est connu comme une plante médicinale diurétique, aromatique, antioxydante et antirhumatismale. Il est largement utilisé dans l'industrie alimentaire comme aromatisant et agent de conservation, en raison de la présence des diterpènes phénoliques, qui ont des propriétés antioxydantes et antimicrobiennes (Zaouali et al., 2012).

(i) Présentation de *Cynara cardunculus*

- **Description botanique**

Cynara cardunculus L. communément appelé « cardon » (figure 12), comprend trois variétés différentes. Cependant, il existe (i) l'artichaut du globe [*C. cardunculus* L. var. *scolymus* (L.) Fiori], (ii) le cardon cultivé [*C. cardunculus* L. var. *altilis*], et (iii)

le cardon sauvage [*C. cardunculus* L. var. *sylvestris*. (Gostin & Waisundara, 2019) Toutes ces espèces appartiennent à la famille des Asteraceae. *Cynara cardunculus*, est une plante tolérante au sel qui pousse naturellement dans des conditions d'habitat difficiles caractérisées par une température élevée, une salinité élevée et une sécheresse en été.



Figure 12 : *C. cardunculus* collecté au stade végétatif dans son environnement naturel située en Tunisie.

- **Intérêts et utilisations**

Cette plante polyvalente est utilisée dans plusieurs plats, comme des soupes et / ou des salades. (Gostin & Waisundara, 2019) Par ailleurs, les fleurs de *C. cardunculus* sont traditionnellement utilisées pour la préparation du fromage (Gostin & Waisundara, 2019) tandis que les feuilles sont particulièrement connues dans le folklore pour leur potentiel thérapeutique en tant que diurétique, cholérétique, cholagogue, antidiabétique et antimicrobien (Ksouri et al., 2012). Elles peuvent être classés comme aliments fonctionnels et la fleur comestible est réputée avoir un

potentiel antimicrobien et anti-VIH, ainsi que pour leurs effets anti-inflammatoires, anti-génétoxiqes et anti-obésité. Ces propriétés essentielles sont liées à leur teneur élevée en composés polyphénoliques. (Rial et al., 2014 Falleh et al., 2008; El Sohaimy, 2014).

3.3 Métabolites secondaires phytotoxiques : Mode d'action et intérêts potentiel dans la gestion des adventices

3.3.1 Généralités

Le mode d'action de certains composés allélochimiques est similaire aux herbicides synthétiques mais beaucoup d'entre eux influencent plusieurs fonctions physiologiques chez la plante. Cela rend la résistance des plantes à ces allélochimiques plus difficiles (Li et al., 2010). Parmi ces composés, les effets phytotoxiques des monoterpénoïdes ont été rapportés (Bouabidi et al., 2015 ; Hazrati et al., 2017 ; Uremis, Arslan, & Sangun, 2009 ; Ben Kaab et al., 2019 ; Alipour & Saharkhiz, 2016) avec des effets phytotoxiques spécifiques comme l'inhibition de la respiration (réduction des mitochondries intracellulaire) et de la photosynthèse, la réduction de la perméabilité des parois cellulaires, aussi bien que l'accélération de la destruction oxydative des pigments chloroplastiques (Barney et al., 2005). Le pinène est l'un des principaux composants volatils émis par une large gamme de plantes aromatiques dont le pouvoir allélochimique est connu. En plus, il a été démontré aussi que l'eugénol (100–1 000 μ M) peut retarder la germination d' *Avena fatua* et fortement inhiber la croissance des racines et des coléoptiles par des altérations physiologiques et biochimiques (Ahuja et al., 2015). L'artémisinine, une lactone sesquiterpène, isolé à partir des tissus de feuilles de *Artemisia* a été rapporté comme un allélochimiques composés qui inhibe l' allongement des racines et pousses de laitue.(Yan et al., 2015).

Dans les cellules eucaryotes, il a été démontré que les huiles essentielles perméabilisaient les membranes externe et mitochondriale, ainsi que l'apoptose et la nécrose (Chaimovitsh et al., 2010). D'autres part, de nombreuses études ont montré que la production d'espèces réactives de l'oxygène (reactive oxygen species ou ROS en anglais) lié au stress oxydatif a été retenue qui comme un des mécanismes majeurs de l'action des phytotoxines. (Kaur et al., 2012a).

3.3.2 Molécules allélochimiques ayant un effet herbicide

De nombreuses plantes ont montré un potentiel allélopathique intéressant. Une partie d'entre-elles sont présentées dans le tableau 5. Par exemple, des exsudats racinaires de l'orge ont montré des effets allélopathiques et d'autotoxicité. En effet, Un grand nombre de métabolites secondaires (phénols, alcaloïdes, cyanoglucosides,

polyamines, etc.) ont été identifiés comme potentiellement allélopathiques chez l'orge. (Lebecque et al., 2018).

Beaucoup de plantes de sorgho sont utilisées en agriculture comme plantes de couverture pour supprimer les adventices. (Uddin et al., 2014) D'ailleurs, Des exsudats des poils racinaires issus des graines de sorgho (*Sorghum bicolor*) contiennent des quinones hydrophobes (sorgoleone) qui sont phytotoxiques pour plusieurs espèces de plantes. Ces molécules peuvent agir en pré et post-émergence et inhiber la croissance de plantes (Soltys, 2013). Les mécanismes d'action pourraient consister en une perturbation dans les fonctions de transport d'électrons dans les mitochondries et les chloroplastes, ce qui inhiberait la croissance des plantes. (Hejl & Koster, 2004).

Des molécules allélochimiques ont été extraites à partir du seigle, *Secale cereale*. Il s'agit du 2(3H)-benzoxazolinone (BOA) et du 2,4-dihydroxy-1,4(2H)-benzoxazin-3-1 (DIBOA). Ces deux molécules inhibent la croissance du concombre (*Cucumis sativus*) par la diminution de la longueur des racines le nombre de racines latérales (Batish., et al 2006) 2003).

D'autres part de nombreuses études ont montré que le riz est une plante allélopathique et libère des substances allélochimiques dans son environnement. Parmi ces substances, des acides phénoliques, des acides gras, des acides phénylalcanoïques, des acides hydroxamiques, des terpènes et des indoles ont été identifiés. Cependant, il a été démontré que les momilactones A et B étaient parmi les composés allélochimiques les plus importants chez le riz. Les momilactones B sont sécrétés par les racines et possèdent des propriétés phytotoxiques. Le taux de sécrétion des momilactones augmente avec la présence de certaines mauvaises herbes et de leur exsudat racinaire (Dayan et al., 2009).

Des composés phénoliques ont été isolés à partir de la luzerne cultivée, *Medicago sativa*, ou alfalfa. Ceux-ci ont des effets sur la croissance et la morphologie racinaire de *Echinochloa crus-galli*. Les composés identifiés sont la coumarine, l'acide cinnamique, l'acide coumarique et l'acide hydro-cynamique (Chon et al., 2005). Des extraits aqueux des feuilles ont un effet d'inhibition sur la croissance racinaire de la luzerne et sur la germination. Les composés suivants ont été identifiés ; coumarine, acide trans-cinnamique, acide coumarique et acide chlorogénique (Chon et al., 2005).

Plusieurs espèces de *Brassicaceae* invasives possèdent un système de défense grâce à des glucosinolates et myrosinase. Après avoir perturbé les tissus, les glucosinolates sont dégradés par des enzymes myrosinases, ce qui résulte en la libération de divers produits d'hydrolyse comme les isothiocyanates, nitriles et autres. Ce système spécifique de défense de la plante peut être un des facteurs clés du succès invasif des *Brassicaceae* (Müller, 2009). Pour cette raison Certaines *Brassicaceae*, comme le colza, peuvent être employées comme plantes de couverture.

Finalement, le tournesol (*Helianthus annuus* L.) est connu pour influencer de manière active la croissance de plantes dans son environnement grâce à son pouvoir allélopathique élevé. Il a été démontré que des extraits de racine du tournesol ont un impact néfaste sur la croissance et la physiologie du blé (*Triticum aestivum*). D'ailleurs, Plus de 200 composés allélopathiques naturels ont été isolés à partir des cultivars du tournesol (Kamal, 2011). Les composés allélochimiques sont principalement des métabolites secondaires et appartiennent, entre autres, aux terpénoïdes, aux composés phénoliques, aux cyanures organiques et aux acides gras à longue chaîne. L'action des substances allélochimiques dans la plante cible est diverse et affecte un grand nombre de réactions biochimiques résultant de modifications de différentes fonctions physiologiques.

Tableau 5 : Molécules allélochimiques libérées par des plantes ayant un effet sur des adventices.

Plantes	Molécules allélochimiques	Modes d'action	Adventices	Références
Orge	Phénols, alcaloïdes, cyanoglucosides, polyamines	Inhibition croissance racicules et coléoptiles	<i>Bromus diandrus</i> et <i>Lolium rigidum</i>	Bouhaouel <i>et al.</i> , 2015
Sorgho (<i>Sorghum bicolor</i>)	Sorgoleone	Effets sur la photosynthèse	<i>Rumex japonicus</i> et <i>Plantago asiatica</i> ,	Hejl et Koster, 2004 ; Uddin <i>et al.</i> , 2014
<i>Croton ciliatoglanduliferus</i>	Retusin pachypodol		<i>Physalis ixocarpa</i> , <i>Trifolium alexandrinum</i> and <i>Lolium perenne</i> . <i>F</i>	Morales-flores, 2015
<i>Eucalyptus kochii</i>	1,8-cineole		<i>Lomatium rigidum</i>	Barton <i>et al.</i> , 2014
Blé (<i>Triticum aestivum</i> L.)	hydroxamique, acide phénoliques et acides gras	Inhibition croissance, système antioxydant	<i>Echinochloa crus galli</i> , <i>L. sativum</i>	Aslam <i>et al.</i> , 2016
Seigle	2(3H)-benzoxazolinone (BOA); 2,4-dihydroxy-1,4(2H)-benzoxazin-3-1 (DIBOA)	Métabolisme des lipides et synthèse des protéines	<i>Cucumis sativus</i>	Burgos <i>et al.</i> , 2003
Sarrasin (<i>Fagopyrum esculentum</i>)	Rutine, quercetine, catechine, epicatechine, acide chlorogénique, acide caféique, acide ferulique et acide gallique.	Expression de gènes	<i>Arabidopsis thaliana</i> L.	Golisz <i>et al.</i> , 2008
Riz (<i>Oryza sativa</i> L.)	momilactones		<i>Echinochloa crus-galli</i>	Kong <i>et al.</i> , 2006 ; Kato-Noguchi et Peters, 2013
Brassicaceae	Glucosinolates	Biosynthèse de protéines	<i>Lolium perenne</i> L., <i>Triticum aestivum</i> L., <i>Lepidium sativum</i> L., <i>Bromus rigidus</i>	Müller, 2009
<i>Pennisetum purpureum</i>	2,4-Di-tert-butylphenol (2,4-DTBP)	Peroxydation lipidique et dommages	<i>Leptochloa chinensis</i> <i>Hedyotis verticillata</i>	Chuah <i>et al.</i> , 2015

<i>Eucalyptus erythrocorys</i>	α -pinène, 1,8-cineole	membranaires	<i>Sinapis arvensis</i> L. et <i>Phalaris canariensis</i> L.	Ghnaya <i>et al.</i> , 2013
<i>Cymbopogon citratus</i>	Citral	Polymérisation des microtubules, intégrité membranaire		Chaimovitsh <i>et al.</i> , 2010
Luzerne cultivée	Coumarine, l'acide cinnamique, l'acide coumarique et l'acide hydro-cynamique	Effets sur croissance et morphologie racinaire	<i>Echinochloa crus-galli</i>	Chon <i>et al.</i> , 2002
Laitue	Coumarine, acide cinnamique, acide coumarique et acide chlorogénique		Alfalfa	Chon <i>et al.</i> , 2005
Tournesol (<i>Helianthus annuus</i> L.)	Phénol	Division cellulaire et processus d'élongation	<i>Triticum aestivum</i> L.	Kamal, 2011

3.3.3 Mode d'action des molécules allélochimiques

3.3.3.1 Membrane et ROS

Un certain nombre de recherches ont été rapportées pour expliquer le mode d'action inhibitrice des métabolites secondaires à la plante. Ces métabolites secondaires de plantes ont été à l'origine de la production excessive des ROS, qui était un facteur important dans le déclenchement de l'effet phytotoxique (Zhi-qiang Yan *et al.*, 2015). En effet, les ROS jouent un rôle de signalisation important pour contrôler un certain nombre de processus essentiels tels que la croissance, le développement, la réponse aux stimuli environnementaux biotiques et abiotiques, la défense contre les agents pathogènes et le comportement stomatique. (Galati *et al.*, 2002). Les ROS peuvent réagir avec des molécules biologiques, telles que l'ADN, des protéines ou des lipides, générant des mutations et des membranes dommageables, entraînant des lésions cellulaires et tissulaires et provoquant des processus de mort cellulaire programmée (PCD) (Mahdavi *et al.*, 2017). Le type principal de ROS est O₂⁻, qui peut être transformé en d'autres ROS nocifs tels que le radical hydroxyle. La production excessive de ROS provoque également des dommages oxydatifs sur les protéines cellulaires, les lipides et les acides nucléiques et active la voie de la mort dans plusieurs types de cellules. (Ksouri *et al.*, 2009). Cependant Hua, *et al.*, 2014 ont montré que le Glaucocalyxin (Gla), extrait de *Isodon japonicus*, augmente le niveau de malondialdéhyde et génère la production de l'anion superoxyde. D'autre part, le mécanisme de phytotoxicité de l'eugénol, un alcool monoterpénoïde, a rapporté

l'altération des mécanismes de défense qui entraîne un retard de croissance des racines. Ceci peut être à l'origine d'une altération de la membrane (une fuite d'électrolyte et une augmentation de malondialdéhyde), la génération d'espèces réactives de l'oxygène (ROS) (Ahuja et al., 2015).

Par ailleurs, d'autres études ont montré l'artémisinine augmente le niveau de MDA et a augmenté chez la laitue. Ceci a été expliqué par le fait qu'une peroxydation lipidique des membranes et des lésions oxydatives ont été provoquées. La rupture des lipides peut modifier la perméabilité, la fluidité des membranes et par conséquent une interférence avec la photosynthèse au niveau de la chaîne de transfert des électrons (Zhi-qiang Yan et al., 2015 ; Batish et al., 2006 ; Hua et al., 2014).

3.3.3.2 Effet sur la division cellulaire

Les composés allélochimiques affectent probablement la division cellulaire et les processus d'élongation au moment de la germination (Yan *et al.*, 2016). Une autre hypothèse liée à la précédente serait une interférence avec des enzymes impliquées dans la mobilisation de nutriments nécessaires à la germination (Kamal, 2011). Une inhibition de la prolifération cellulaire dans les méristèmes apicaux a été postulée comme une des raisons de la division cellulaire et l'inhibition de la croissance (Kaur *et al.*, 2010). Il a été démontré aussi que la mitose peut être retardée dans l'apex racinaire et par conséquent la division cellulaire dans les zones méristématiques et l'élongation des racines seront inhibés (Singh *et al.*, 2006, Yan *et al.*, 2016). Lors de la germination, l' α -amylase joue un rôle majeur dans la dégradation des réserves de carbohydrates en sucres solubles. Dans ce contexte, certaines huiles essentielles inhibent l'activité enzymatique de l' α -amylase (Poonpaiboonpipat et al., 2013). Le potentiel allélopathique de ces molécules est basé sur leurs interactions avec des protéines et des acides aminés qui jouent un rôle important dans le processus de la division cellulaire. Ainsi l'interférence avec la formation de sucres phosphorylés est responsable de l'effet d'inhibition de la division cellulaire, le retard de la croissance racinaire (Müller, 2009).

3.3.3.3 Effet sur le contenu en chlorophylle

Les chlorophylles sont des pigments spécifiques majeurs pour les plantes. Contenus dans les chloroplastes, ils capturent l'énergie lumineuse (Poonpaiboonpipat *et al.*, 2013). De nombreuses études ont montré que le contenu en chlorophylle a et b diminue avec la concentration en extrait végétal (Deng, et al 2014 ; Morales et al., 2015 ; Radhakrishnan et al., 2018) Cela indique que l'extrait interfère avec le métabolisme photosynthétique. (Kaur *et al.*, 2010).

Une réduction dans l'assimilation de CO₂ a été observée dans des plantes traitées avec des composés allélochimiques. Ces derniers affecteraient la photosynthèse en inhibant le transport d'électrons dans le photosystème II (PSII) (M. Liu & Lu, 2016). Une autre étude a montré que le sorgoleone, un composé allélochimique, viendrait se lier au site de fixation d'une quinone (QB) du PSII. Le sorgoleone inhibe l'activité d'une enzyme nécessaire à la synthèse des caroténoïdes qui inclut des précurseurs de plastoquinone (Hejl & Koster, 2004).

3.3.3.4 Effet sur la respiration cellulaire

Les mitochondries, sites de la respiration cellulaire, peuvent être considérées comme un site potentiel d'action allélochimique (Ahuja et al., 2015). En effet, Les composés allélochimiques tels que des acides phénoliques, des quinones, des coumarines, des terpénoïdes et des flavonoïdes ont montré avoir un effet sur l'activité respiratoire de tissus de plantes (Hejl & Koster, 2004). Les processus affectés peuvent être le flux d'électrons mitochondriaux, la réduction de l'oxygène, la phosphorylation oxydative, le prélèvement en phosphate, l'activité de la NADH-déshydrogénase (Reigosa et al., 2006). Kaur et al., 2012 ont pu observer une accumulation de globules lipidiques dans le cytoplasme et une réduction de la taille d'organites telles que les mitochondries dans les plantes traitées avec l'HE de *Artemisia scoparia*. Ce phénomène serait probablement dû à une inhibition de la synthèse d'ADN ou une perturbation des membranes. Par ailleurs, (Burgos et al., 2004) ont remarqué une diminution du nombre de mitochondries causée par deux molécules allélochimiques qui sont le BOA et DIBOA.

3.3.3.5 Induction de stress oxydatifs

Récemment, il a été mis en évidence que des composés allélochimiques puissent causer des stress oxydatifs à travers la production d'espèces réactives de l'oxygène (ROS). Ces molécules sont, comme leur nom l'indique, fortement réactives mais également toxiques et peuvent causer des dommages oxydatifs aux membranes, à l'ADN, aux protéines, aux pigments photosynthétiques et aux lipides (Singh *et al.*, 2006). Par exemple, L'eugénol sous un stress oxydatif, peut mener à une surproduction de ROS menant à la toxicité de la plante. Cette génération excessive de ROS endommage des biomolécules majeures (protéines, lipides et ADN), perturbe l'intégrité membranaire et mène finalement à la mort programmée des cellules (Ahuja *et al.*, 2015). Des enzymes antioxydantes sont activées suite à cette production de ROS. Ces enzymes fournissent une protection secondaire contre les stress oxydatifs. Elles sont impliquées dans la détoxification des ROS (Mahdavia et al., 2017). Le

stress causé par ces molécules allélochimiques produit un déséquilibre oxydatif puissant et par conséquent, une altération de l'activité des enzymes antioxydantes. Le stress oxydatif serait par conséquent un des mécanismes, parmi d'autres, par lequel une plante allélopathique cause une phytotoxicité à une autre plante (Ortega al., 2007).

Deux sites sensible à la formation des ROS qui sont très riches en oxygène réduit. Ces sites sont les chloroplastes au niveau photosystèmes II et I, 2015 et les mitochondries (Chuah & Ismail, 2015 ; Ishii-Iwamoto *et al.*, 2006).

3.3.3.6 Induction d'une peroxydation lipidique

La peroxydation des lipides est une dégradation oxydative des lipides (composants structurels des membranes) perturbant l'intégrité membranaire et la phosphorylation oxydative, et inhibant la chaîne de transfert des électrons (Ben Ghnaya *et al.*, 2013). En effet La génération d'espèces réactives de l'oxygène résulte cette peroxydation lipidique ainsi que des dégâts dans les membranes des tissus racinaires et dans les chloroplastes des tissus foliaires (Kaur *et al.*, 2010 ; Chuah *et al.*, 2015).

3.3.3.7 Effet sur des enzymes membranaires

La pompe H^+ - ATPase et d'autres protéines membranaires associées jouent un rôle essentiel dans la maintenance de la turgescence cellulaire et le prélèvement de minéraux essentiels à la croissance de la plante. Tout changement dans l'activité de H^+ - ATPase mènerait à la fermeture des stomates, diminuerait la turgescence des feuilles et aurait un effet indirect sur d'autres fonctions essentielles de la plante telles que la photosynthèse. L'inhibition du prélèvement en ions serait également causée par une perturbation membranaire (Zhou et Yu, 2006). Une altération dans l'absorption de l'eau régulée par les pompes à protons à travers les plasmalemmes des cellules racinaires causerait de multiples conséquences physiologiques telles qu'une croissance ralentie de la plante (Hejl et Koster, 2004). Dans ce contexte, un niveau de peroxydation lipidique élevé peut être mis en relation avec l'inhibition de deux enzymes associées aux membranes, H^+ -ATPase et NADPH oxydase (Cruz-Ortega *et al.*, 2007). Cet effet d'inhibition serait le résultat de plusieurs processus métaboliques affectés à différents moments. En effet, plusieurs études ont montré que des composés contenus dans des HE traverseraient les membranes cellulaires, interagissant avec des enzymes et des protéines des membranes telles que la pompe membranaire H^+ -ATPase, produisant un flux de protons vers l'extérieur qui induirait des changements dans les cellules, et finalement, leur mort (Ben Ghnaya *et al.*, 2013).

4. Références

- Ahuja, N., Batish, D. R., Singh, H. P., & Kohli, R. K. (2015). Herbicidal activity of eugenol towards some grassy and broad-leaved weeds. *Journal of Pest Science*, 88(1), 209–218. <https://doi.org/10.1007/s10340-014-0570-x>
- Ahuja, N., Pal, H., Rani, D., & Kumar, R. (2015). Eugenol-inhibited root growth in *Avena fatua* involves ROS-mediated oxidative damage. *Pesticide Biochemistry and Physiology*, 118, 64–70. <https://doi.org/10.1016/j.pestbp.2014.11.012>
- Ajila, C. M., Brar, S. K., Verma, M., Tyagi, R. D., Godbout, S., & Valéro, J. R. (2011). Extraction and Analysis of Polyphenols: Recent trends. *Critical Reviews in Biotechnology*, 31(3), 227–249. <https://doi.org/10.3109/07388551.2010.513677>
- Alipour, M., & Saharkhiz, M. J. (2016). Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phenological growth stages. *Biocatalysis and Agricultural Biotechnology*, 7, 271–278. <https://doi.org/10.1016/j.bcab.2016.07.003>
- Arceusz, A., Wesolowski, M., & Konieczynski, P. (2013). Methods for extraction and determination of phenolic acids in medicinal plants: A review. *Natural Product Communications*, 8(12), 1821–1829. <https://doi.org/10.1177/1934578x1300801238>
- Asbahani, A. El, Miladi, K., Badri, W., Sala, M., Addi, E. H. A., Casabianca, H., Mousadik, A. El, Hartmann, D., Jilale, A., Renaud, F. N. R., & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International Journal of Pharmaceutics*, 483(1–2), 220–243. <https://doi.org/10.1016/j.ijpharm.2014.12.069>
- Bailey, K. L. (2013). The Bioherbicide Approach to Weed Control Using Plant Pathogens. In *Integrated Pest Management: Current Concepts and Ecological Perspective* (Issue 1977). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-398529-3.00014-2>
- Bajwa, A. A., Mahajan, G., & Chauhan, B. S. (2015). Nonconventional Weed Management Strategies for Modern Agriculture. *Weed Science*, 63(04), 723–747. <https://doi.org/10.1614/ws-d-15-00064.1>
- Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - A review. *Food and Chemical Toxicology*, 46(2), 446–475. <https://doi.org/10.1016/j.fct.2007.09.106>
- Barney, J. N., Hay, A. G., & Weston, L. A. (2005). Isolation and characterization of allelopathic volatiles from mugwort (*Artemisia vulgaris*). *Journal of Chemical*

- Ecology*, 31(2), 247–265. <https://doi.org/10.1007/s10886-005-1339-8>
- Batish, D. R., Singh, H. P., Setia, N., Kaur, S., & Kohli, R. K. (2006). 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). 44, 819–827. <https://doi.org/10.1016/j.plaphy.2006.10.014>
- Ben Kaab, S., Rebey, I. B., Hanafi, M., Berhal, C., Fauconnier, M. L., De Clerck, C., Ksouri, R., & Jijakli, H. (2019). Rosmarinus officinalis essential oil as an effective antifungal and herbicidal agent. *Spanish Journal of Agricultural Research*, 17(2), e1006. <https://doi.org/10.5424/sjar/2019172-14043>
- Böcker, T., Möhring, N., & Finger, R. (2019). Herbicide free agriculture? A bio-economic modelling application to Swiss wheat production. *Agricultural Systems*, 173(March), 378–392. <https://doi.org/https://doi.org/10.1016/j.agsy.2019.03.001>
- Bouabidi, W., Hanana, M., Gargouri, S., Amri, I., Fezzani, T., Ksontini, M., Jamoussi, B., & Hamrouni, L. (2015). Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis* L. essential oils. *Natural Product Research*, 29(9), 864–868. <https://doi.org/10.1080/14786419.2014.980246>
- Burgos, N. R., Talbert, R. E., Kim, K. S., & Kuk, Y. I. (2004). Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye (*Secale cereale*). *Journal of Chemical Ecology*, 30(3), 671–689. <https://doi.org/10.1023/B:JOEC.0000018637.94002.ba>
- Chaimovitsh, D., Abu-abied, M., Belausov, E., Rubin, B., Dudai, N., & Sadot, E. (2010). Microtubules are an intracellular target of the plant terpene citral. 399–408. <https://doi.org/10.1111/j.1365-313X.2009.04063.x>
- Chengxu, W., Mingxing, Z., Xuhui, C., & Bo, Q. (2011). Review on Allelopathy of Exotic Invasive Plants. *Procedia Engineering*, 18, 240–246. <https://doi.org/10.1016/j.proeng.2011.11.038>
- Chon, S., Choi, S., Jung, S., & Jang, H. (2002). Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. 21(0261), 1077–1082.
- Chuah, T. S., & Ismail, B. S. (2015). *Evaluation of the biochemical and physiological activity of the natural compound, 2, 4-ditert-butylphenol on weeds*. 214–223.
- Cordeau, S., Triolet, M., Wayman, S., Steinberg, C., & Guillemin, J. P. (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. *Crop Protection*, 87, 44–49. <https://doi.org/10.1016/j.cropro.2016.04.016>

- Creech, C. F., Henry, R. S., Hewitt, A. J., & Kruger, G. R. (2018). Herbicide Spray Penetration into Corn and Soybean Canopies Using Air-Induction Nozzles and a Drift Control Adjuvant. *Weed Technology*, 32(1), 72–79. <https://doi.org/10.1017/wet.2017.84>
- Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). (*Secondary Metabolites*). 1250–1318.
- Davis, A. S., & Frisvold, G. B. (2017). Are herbicides a once in a century method of weed control? *Pest Management Science*, 73(11), 2209–2220. <https://doi.org/10.1002/ps.4643>
- Dayan, F. E., Cantrell, C. L., & Duke, S. O. (2009). Natural products in crop protection. *Bioorganic and Medicinal Chemistry*, 17(12), 4022–4034. <https://doi.org/10.1016/j.bmc.2009.01.046>
- Dayan, F. E., Owens, D. K., & Duke, S. O. (2012). Rationale for a natural products approach to herbicide discovery. *Pest Management Science*, 68(4), 519–528. <https://doi.org/10.1002/ps.2332>
- Deng, C., Shao, H., Pan, X., Wang, S., & Zhang, D. (2014). Herbicidal effects of harmaline from *Peganum harmala* on photosynthesis of *Chlorella pyrenoidosa* : Probed by chlorophyll fluorescence and thermoluminescence. *Pesticide Biochemistry and Physiology*, 115, 23–31. <https://doi.org/10.1016/j.pestbp.2014.08.002>
- El Sohaimy, S. a. (2014). Chemical Composition, Antioxidant and Antimicrobial Potential of Artichoke. *The Open Nutraceuticals Journal*, 1(1), 15–20. <https://doi.org/10.2174/1876396001407010015>
- Ewané, C. A., Lepoivre, P., Lassois, L., & de Bellaire, L. de L. (2012). Involvement of phenolic compounds in the susceptibility of bananas to crown rot. A review. *Biotechnology, Agronomy and Society and Environment*, 16(3), 393–404.
- Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., & Abdelly, C. (2008). Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Comptes Rendus - Biologies*, 331(5), 372–379. <https://doi.org/10.1016/j.crv.2008.02.008>
- Galati, G., Sabzevari, O., Wilson, J. X., & Brien, P. J. O. (2002). Prooxidant activity and cellular effects of the phenoxy radicals of dietary flavonoids and other polyphenolics. *177*, 91–104.
- Gostin, A. I., & Waisundara, V. Y. (2019). Edible flowers as functional food: A review on artichoke (*Cynara cardunculus* L.). *Trends in Food Science and Technology*, 86, 381–391. <https://doi.org/10.1016/j.tifs.2019.02.015>

- Hazrati, H., Saharkhiz, M. J., Niakousari, M., & Moein, M. (2017). Natural herbicide activity of *Satureja hortensis* L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. *Ecotoxicology and Environmental Safety*, 142(April), 423–430. <https://doi.org/10.1016/j.ecoenv.2017.04.041>
- Heap, I. (2014). Global perspective of herbicide-resistant weeds. *Pest Management Science*, 70(9), 1306–1315. <https://doi.org/10.1002/ps.3696>
- Hejl, A. M., & Koster, K. L. (2004). Juglone disrupts root plasma membrane h - atpase activity and impairs water uptake , root respiration , and growth in soybean (*Glycine max*) and corn (*Zea mays*). 30(2), 453–471.
- Heleno, S. A., Martins, A., João, M., Queiroz, R. P., & Ferreira, I. C. F. R. (2015). Bioactivity of phenolic acids : Metabolites versus parent compounds : A review. *Food chemistry*, 173, 501–513. <https://doi.org/10.1016/j.foodchem.2014.10.057>
- Hua, W., Li, Y., Zheng, P., Yan, H., & Wang, J. W. (2014). Glaucocalyxin A and B Regulate Growth and Induce Oxidative Stress in Lettuce (*Lactuca sativa* L .) Roots. 384–396. <https://doi.org/10.1007/s00344-013-9391-z>
- Jabran, K., Mahajan, G., Sardana, V., & Chauhan, B. S. (2015). Allelopathy for weed control in agricultural systems. *Crop Protection*, 72, 57–65. <https://doi.org/10.1016/j.cropro.2015.03.004>
- Kamal, J. (2011). Impact of allelopathy of sunflower (*Helianthus annuus* L .) roots extract on physiology of wheat (*Triticum aestivum* L .). 10(65), 14465–14477. <https://doi.org/10.5897/AJB11.988>
- Kaur, S., Singh, H. P., Batish, D. R., & Kohli, R. K. (2012). Artemisia scoparia essential oil inhibited root growth involves reactive oxygen species (ROS)-mediated disruption of oxidative metabolism: In vivo ROS detection and alterations in antioxidant enzymes. *Biochemical Systematics and Ecology*, 44, 390–399. <https://doi.org/10.1016/j.bse.2012.06.015>
- Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, 18(2), 2328–2375. <https://doi.org/10.3390/molecules18022328>
- Koul, O., Walia, S., & Dhaliwal, G. S. (2008). Essential oils as green pesticides: Potential and constraints. *Biopesticides International*, 4(1), 63–84.
- Ksouri, R., Ksouri, W. M., Jallali, I., Debez, A., Hiroko, I., Abdelly, C., Ksouri, R., Ksouri, W. M., Jallali, I., Debez, A., & Magné, C. (2012). Medicinal halophytes : potent source of health promoting biomolecules with medical , nutraceutical and food applications Medicinal halophytes : potent source of

- health promoting biomolecules with medical , nutraceutical and food applications. *8551*(January 2017).
<https://doi.org/10.3109/07388551.2011.630647>
- Lebecque, S., Crowet, J., Lins, L., Delory, B. M., Patrick, J., Fauconnier, M., & Deleu, M. (2018). Interaction between the barley allelochemical compounds gramine and hordenine and artificial lipid bilayers mimicking the plant plasma membrane. *November 2017*, 1–13. <https://doi.org/10.1038/s41598-018-28040-6>
- Li, Z.-H., Wang, Q., Ruan, X., Pan, C.-D., & Jiang, D.-A. (2010). Phenolics and Plant Allelopathy. *Molecules*, *15*(12), 8933–8952.
<https://doi.org/10.3390/molecules15128933>
- Liu, M., & Lu, S. (2016). Plastoquinone and Ubiquinone in Plants: Biosynthesis, Physiological Function and Metabolic Engineering. *Frontiers in Plant Science*, *7*(December), 1–18. <https://doi.org/10.3389/fpls.2016.01898>
- Liu, Q., Wu, C., Peng, A., Gao, K., Chen, J., Li, Y., & Fu, H. (2017). Flavonolignans from *Elymus natans* L. and Phytotoxic Activities. *Journal of Agricultural and Food Chemistry*, *65*(7), 1320–1327.
<https://doi.org/10.1021/acs.jafc.6b04162>
- Mahdavia, F., Saharkhiz, M. J., & Karami, A. (2017). Defensive response of radish seedlings to the oxidative stress arising from phenolic compounds in the extract of peppermint (*Mentha × piperita* L.). *Scientia Horticulturae*.
<https://doi.org/10.1016/j.scienta.2016.11.029>
- Melander, B., Rasmussen, I. A., & Bärberi, P. (2005). Integrating physical and cultural methods of weed control— examples from European research. *Weed Science*, *53*(3), 369–381. <https://doi.org/10.1614/ws-04-136r>
- Morales-flores, F., Olivares-palomares, K. S., Rivero-cruz, J. F., Lotina-hennsen, B., & King-d, B. (2015). *Flavonoids Affect the Light Reaction of Photosynthesis in Vitro and in Vivo as Well as the Growth of Plants*.
<https://doi.org/10.1021/acs.jafc.5b02842>
- Nostro, A., Germanò, M. P., D’Angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology*, *30*(5), 379–384.
<https://doi.org/10.1046/j.1472-765x.2000.00731.x>
- Pavela, R., & Benelli, G. (2016). Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. *Trends in Plant Science*, *21*(12), 1000–1007.
<https://doi.org/10.1016/j.tplants.2016.10.005>

- Poonpaiboonpipat, T., Pangnakorn, U., Suvunnamek, U., Teerarak, M., Charoenying, P., & Laosinwattana, C. (2013). Phytotoxic effects of essential oil from *Cymbopogon citratus* and its physiological mechanisms on barnyardgrass (*Echinochloa crus-galli*). *Industrial Crops and Products*, 41(1), 403–407. <https://doi.org/10.1016/j.indcrop.2012.04.057>
- Radhakrishnan, R., Alqarawi, A. A., & Abd Allah, E. F. (2018). Bioherbicides: Current knowledge on weed control mechanism. *Ecotoxicology and Environmental Safety*, 158(April), 131–138. <https://doi.org/10.1016/j.ecoenv.2018.04.018>
- Rehman, R., Hanif, M. A., Mushtaq, Z., & Al-Sadi, A. M. (2016). Biosynthesis of essential oils in aromatic plants: A review. *Food Reviews International*, 32(2), 117–160. <https://doi.org/10.1080/87559129.2015.1057841>
- Reigosa, M. J. (n.d.). *Allelopathy: A Physiological Process*.
- Reyes-Jurado, F., Franco-Vega, A., Ramírez-Corona, N., Palou, E., & López-Malo, A. (2015). Essential Oils: Antimicrobial Activities, Extraction Methods, and Their Modeling. *Food Engineering Reviews*, 7(3), 275–297. <https://doi.org/10.1007/s12393-014-9099-2>
- Rial, C., Novaes, P., Varela, R. M., Molinillo, J. M. G., & Macias, F. A. (2014). Phytotoxicity of Cardoon (*Cynara cardunculus*) Allelochemicals on Standard Target Species and Weeds.
- Sbai, H., Saad, I., Ghezal, N., Greca, M. Della, & Haouala, R. (2016). Bioactive compounds isolated from *Petroselinum crispum* L. leaves using bioguided fractionation. *Industrial Crops and Products*, 89, 207–214. <https://doi.org/10.1016/j.indcrop.2016.05.020>
- Shariq I. Sherwani, I. A. A. and H. A. K. (2016). We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists TOP 1%. *Intech, i(tourism)*, 13. <https://doi.org/http://dx.doi.org/10.5772/57353>
- Soltys, D., Krasuska, U., Bogatek, R., & Gniazdowska, a. (2013). Allelochemicals as Bioherbicides - Present and Perspectives. *Herbicides - Current Research and Case Studies in Use*, 517–542. <https://doi.org/10.5772/56185>
- Taylor, P., Street, M., Wt, L., Cruz-ortega, R., Lara-núñez, A., Anaya, A. L., Cruz-ortega, R., Lara-núñez, A., & Anaya, A. L. (2007). Allelochemical Stress Can Trigger Oxidative Damage in Receptor Plants and es io s ci en ce . *D r u. May 2015*, 3–5. <https://doi.org/10.4161/psb.2.4.3895>
- Tesio, F., & Ferrero, A. (2010). Allelopathy, a chance for sustainable weed management. *International Journal of Sustainable Development and World Ecology*, 17(5), 377–389. <https://doi.org/10.1080/13504509.2010.507402>

- Tohidi, B., Rahimmalek, M., & Trindade, H. (2019). Review on essential oil, extracts composition, molecular and phytochemical properties of *Thymus* species in Iran. *Industrial Crops and Products*, 134(February), 89–99. <https://doi.org/10.1016/j.indcrop.2019.02.038>
- Uddin, M. R., Park, S. U., Dayan, F. E., & Pyon, J. Y. (2014). Herbicidal activity of formulated sorgoleone, a natural product of sorghum root exudate. *Pest Management Science*, 70(2), 252–257. <https://doi.org/10.1002/ps.3550>
- Uremis, I., Arslan, M., & Sangun, M. K. (2009). Herbicidal activity of essential oils on the germination of some problem weeds. *Asian Journal of Chemistry*, 21(4), 3199–3210.
- van Evert, F. K., Fountas, S., Jakovetic, D., Crnojevic, V., Travlos, I., & Kempenaar, C. (2017). Big Data for weed control and crop protection. *Weed Research*, 57(4), 218–233. <https://doi.org/10.1111/wre.12255>
- Varanasi, V. K., Godar, A. S., Currie, R. S., Dille, A. J., Thompson, C. R., Stahlman, P. W., & Jugulam, M. (2015). Field-evolved resistance to four modes of action of herbicides in a single kochia (*Kochia scoparia* L. Schrad.) population. *Pest Management Science*, 71(9), 1207–1212. <https://doi.org/10.1002/ps.4034>
- Ve, C. (2012). Phenolic compounds : from plants to foods. *000*, 153–177. <https://doi.org/10.1007/s11101-012-9242-8>
- Vyvyan, J. R. (2002). Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron*, 58(9), 1631–1646. [https://doi.org/10.1016/S0040-4020\(02\)00052-2](https://doi.org/10.1016/S0040-4020(02)00052-2)
- Wang, C. J., & Liu, Z. Q. (2007). Foliar uptake of pesticides-Present status and future challenge. *Pesticide Biochemistry and Physiology*, 87(1), 1–8. <https://doi.org/10.1016/j.pestbp.2006.04.004>
- Weir, T. L., Park, S.-W., & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology*, 7(4), 472–479. <https://doi.org/10.1016/j.pbi.2004.05.007>
- Yan, Z. Q., Wang, D. D., Ding, L., Cui, H. Y., Jin, H., Yang, X. Y., Yang, J. S., & Qin, B. (2015). Mechanism of artemisinin phytotoxicity action: Induction of reactive oxygen species and cell death in lettuce seedlings. *Plant Physiology and Biochemistry*, 88, 53–59. <https://doi.org/10.1016/j.plaphy.2015.01.010>
- Yan, Z., Wang, D., Ding, L., Cui, H., Jin, H., Yang, X., Yang, J., & Qin, B. (2015). Plant Physiology and Biochemistry Mechanism of artemisinin phytotoxicity action : Induction of reactive oxygen species and cell death in lettuce seedlings.

Plant Physiology et Biochemistry, 88, 53–59.
<https://doi.org/10.1016/j.plaphy.2015.01.010>

Zaouali, Y., Chograni, H., Trimech, R., & Boussaid, M. (2012). Genetic diversity and population structure among *Rosmarinus officinalis* L. (Lamiaceae) varieties: Var. *typicus* Batt. and var. *trogodytorum* Maire. based on multiple traits. *Industrial Crops and Products*, 38(1), 166–176.
<https://doi.org/10.1016/j.indcrop.2012.01.011>

2

Objectifs généraux de la thèse

Objectifs généraux et choix expérimentaux de la thèse

En analysant l'état de l'art concernant l'utilisation des extraits végétaux dans la lutte contre les adventices, nous avons pu remarquer que beaucoup d'études portaient seulement sur la présence ou l'absence de l'efficacité et la détermination des concentrations minimales et maximales d'inhibition de la croissance. Nous avons donc décidé d'aller plus loin dans la démarche et d'étudier le mode d'action de ces extraits végétaux et voir la possibilité de formuler un bioherbicide efficace. Dans un premier temps, le choix des plantes a constitué une étape difficile de cette thèse. Ceci a été réalisé en se référant à la littérature. En effet 6 plantes halophytes à savoir *Limoniastrum guyonianum*, *Nitraria retusa*, *Cynara cardunculus*, *Artemisia herba-alba*, *Mesembryanthemum edule*, *Tamarix gallica*, ont été sélectionnées pour leur teneur, en composés phénoliques. Ensuite, *R. officinalis* a été choisie pour son rendement en huile essentielle et ses nombreuses activités biologiques. Les autres plantes ont été collectées du fait de leurs effets thérapeutiques intéressants et leurs utilisations dans la médecine préventive. Dans un deuxième temps, nous avons suivi un protocole expérimental d'extraction pour toutes les plantes et un protocole d'identification pour la plante la plus prometteuse qui ciblait les composés phénoliques dont l'étude de l'activité herbicide a été peu étudiée. Après avoir retenu 2 bons candidats, une formulation a été élaborée en fonction de l'aspect physicochimique de chaque extrait de plante.

Le choix du modèle expérimental a également soulevé des difficultés. En effet, les essais de pré-émergence classique étaient très étudiés dans la littérature. Pour cela, nous avons mis au point un nouveau protocole pour tester l'activité herbicide des extraits végétaux en post-émergence. Plusieurs solutions ont été pulvérisées dont deux herbicides commerciaux.

Finalement, le mode d'action des extraits sur la plante cible constitue un volet essentiel pour améliorer nos formulations. Quelques paramètres physiologiques et biochimiques ont été mesurés afin de comprendre l'effet de cet extrait formulé. Par la suite l'étude de l'interaction des molécules actives avec les lipides membranaires était essentielle pour poser des nouvelles hypothèses sur le mode d'action général de cet extrait.

D'une manière plus générale et simple, notre étude vise à développer un bioherbicide à base d'extraits végétaux en tenant compte des composantes principales citées dans la figure 13 :

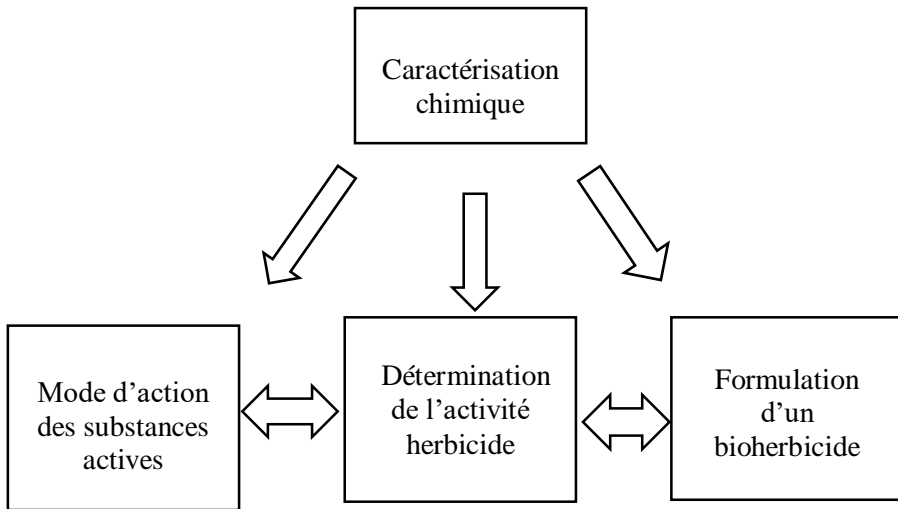


Figure 13 : Objectifs généraux de la thèse.

**Etude du potentiel herbicide des
extraits végétaux (*Cynara
cardunculus*, *Rosmarinus officinalis*)**

La première partie de ce chapitre s'intéresse à la détermination du potentiel herbicide de 10 extraits végétaux pour lutter contre les plantes indésirables et plus particulièrement contre *Trifolium incarnatum* (*T. incarnatum*), *Sylibum marianum* (*S. marianum*) et *Phalaris minor* (*P. minor*). Parmi ces 10 extraits, un seul extrait de celui de *C. cardunculus* est considéré comme candidat potentiel pour le développement d'un bioherbicide à base de plante. D'autres part, il a été confirmé que l'extrait méthanolique de *Cynara cardunculus* inhibait la germination des mauvaises herbes et la croissance des plantules, et provoquait des nécroses et des chloroses. Suite à un fractionnement bio-guidé, cinq principaux composés phénoliques ont été identifiés dans l'extrait méthanolique de *Cynara cardunculus* à savoir: 1) l'acide syringique, 2) l'acide p-coumarique, 3) la myricitrine, 4) la quercétine et 5) la naringénine. Ensuite, seuls 3 flavonoïdes contenus dans la fraction la plus active ont été testés sur *Trifolium incarnatum*. Les 3 composés ont un effet phytotoxique significatif et pourraient donc être utilisés dans une nouvelle composition d'herbicides botaniques pour lutter contre les mauvaises herbes. En outre, une nouvelle composition herbicide a été conçue pour améliorer l'activité de l'extrait méthanolique en post-émergence. La formulation contenant l'extrait méthanolique brut de *C. cardunculus* présente la même activité herbicide que le bioherbicide industriel standard contenant de l'acide pélagonique. Ces résultats font de *C. cardunculus* une source appropriée de composés naturels potentiellement utilisables comme herbicide naturel.

La deuxième partie de ce chapitre s'intéresse à l'étude de l'effet herbicide de l'huile essentielle de *Rosmarinus officinalis* sur les adventices. En effet, afin de réduire l'utilisation de pesticides chimiques, un grand intérêt a été porté aux agents de lutte biologique et aux produits botaniques respectueux de l'environnement qui préservent la biodiversité. Dans ce contexte, cette étude visait à évaluer les activités antifongiques et herbicides de l'huile essentielle de *Rosmarinus officinalis* (OE) afin de trouver une alternative aux pesticides synthétiques. La composition chimique de l'huile essentielle de *Rosmarinus officinalis* a été déterminée par chromatographie en phase gazeuse et spectrométrie de masse (GC-MS). Les résultats ont montré que l'huile essentielle de *R. officinalis* était riche en monoterpènes et que ses composés majoritaires étaient le 1,8-cinéole (54,60%), le camphre (12,27%) et l' α -pinène (7,09%). Cependant, les effets de l'HE sur la croissance des plantules

de mauvaises herbes ont été évalués. Les résultats obtenus ont confirmé que cette HE ralentit la croissance des plantules de *Trifolium incarnatum*, *Silybum marianum* et *Phalaris minor*. En fait, l'HE à 5 mM inhibe complètement la germination des graines. Une autre expérience a été réalisée pour évaluer l'activité herbicide en post-émergence et une nouvelle formulation d'herbicide a été mise en place pour la première fois afin d'améliorer l'activité de l'huile essentielle de *R. officinalis* en post-levée.

Screening of Tunisian plant extracts for herbicidal activity and formulation of a bioherbicide based on *Cynara cardunculus*

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Highlights

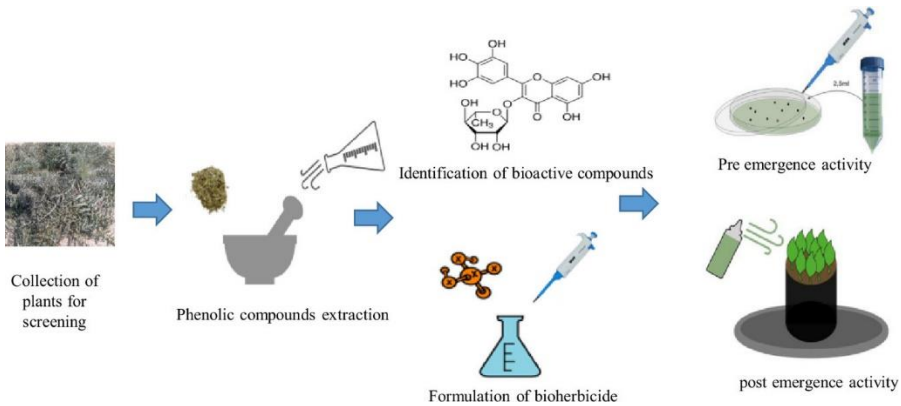
- *C. cardunculus* extract had the best herbicidal activity among 10 plant extracts.
- Five phenolic compounds were identified in *C. cardunculus*.
- Myricitrin, naringenin and quercetin were the main active compounds in *C. cardunculus*
- Fraction 2 of *C. cardunculus* had the same herbicidal effect as biological herbicide.

ABSTRACT

The need to reduce the use of chemical pesticides currently fosters great interest in eco-friendly biological control agents. In addition, the isolation of plant allelopathic substances and the evaluation of their phytotoxic effects can lead to the discovery of new natural herbicides. In this context, our study aimed to assess the herbicidal activity of ten crude extracts obtained from aerial parts of Tunisian spontaneous plants against *Trifolium incarnatum*, *Silybum marianum* and *Phalaris minor*. It confirmed that the *Cynara cardunculus* methanolic extract best inhibited weed germination and seedling growth, and caused necrosis or chlorosis. Following a bioassay-guided fractionation, five main phenolic compounds, (1) syringic acid, (2) *p*-coumaric acid, (3) myricitrin, (4) quercetin and (5) naringenin were identified in the most active crude methanolic extract. Then, only 3 of the flavonoids contained in the most active fraction were tested on *Trifolium incarnatum*. The 3 compounds had a significant phytotoxic effect and therefore could be employed in a new composition of botanical herbicides to control crop weeds. Besides, a novel herbicide composition was designed to improve the post-emergence activity of the methanolic extract. The formulation containing the *C. cardunculus* crude methanolic extract showed the same herbicidal activity as the standard industrial bioherbicide containing pelargonic acid. These results make *C. cardunculus* a suitable source of natural compounds potentially usable as natural herbicides.

Key words: herbicidal activity, extracts, formulation, phenolic compound, bioherbicide

Graphical abstract



1. Introduction

Weeds are registered as harmful plant pests posing a serious problem in agriculture worldwide (Suksungworn et al., 2016). They cause huge economic losses which can rise up to 34% in major crops by affecting yields and competing with crops for nutrients, light, and water (Araniti et al., 2015; Jabran et al., 2015).

The most efficient weed control methods currently include mechanical or hand weeding, and application of chemical herbicides. The latter has been proven to have negative impacts on environmental, animal, and human health (Böcker et al, 2019). Besides, it can increase weed resistance to phytochemicals (Jabran et al., 2015; Ahmed et al., 2017). For these reasons, scientists are working on the identification of a biological solution that can minimize the impacts of synthetic herbicides in agricultural production (Morra et al., 2018; Sbai et al., 2016; Chengxu et al., 2011). This solution could offer a number of benefits such as increased target specificity and rapid degradation of the active substance (Cordeau et al., 2016).

Allelopathic compounds released by plant organs in interaction with the environment exhibit several biological activities, and some of them could be integrated in weed management (Schleiden et al., 2019). They are generally derived from secondary pathways and have been investigated as allelochemicals with allelopathic effects on plants. For these reasons, a huge interest has been focused on plant extracts as sources of allelochemicals used

for weed management (Cordeau et al., 2016). For example, Li et al. (2010), Javaid et al. (2010), Yan et al. (2014), Araniti et al. (2014), Nebo et al. (2014), Ben El Hadj Ali et al. (2014), Ribeiro et al. (2015), Araniti et al. (2015), and Lim et al. (2017) showed that these plant extracts inhibited weed germination and seedling growth. Nevertheless, few studies have shown an herbicidal effect of these compounds in post emergence by direct spraying on weeds. This inhibitory effect has often been related to the presence of phenolic compounds (Omezzine et al., 2011; Sbai et al., 2016; Jelassi et al., 2016). These are the most represented secondary metabolites implied in plant allelopathic compounds (Vyvyan, 2002; Dayan et al., 2012). They are among the most important groups of antioxidant substances, are produced by plants for protection against UV light, insects, and plant pathogens such as viruses (Medini et al., 2014), bacteria (Trabelsi et al., 2013) and fungi (Ksouri et al., 2012; Heleno et al., 2015; Ben Kaab et al., 2019).

The identification of these phenolic compounds and the study of their toxic effect on plants can lead to the development of new nature-based herbicides (Flamini, 2012; Dayan et al., 2012; Cordeau et al., 2016). In this context, our study aims to assess the herbicidal effect of different Tunisian plant extracts and identify the most active one, and to find out the bioactive compounds contained in that extract, so as to design a formulation based on this plant extract.

2. Materials and methods

2.1. Plant material, extraction and fractionation procedures

Aerial parts of ten wild Tunisian spontaneous plants were collected in their vegetative stage on February 2015 from different Tunisian regions that belong to various bioclimatic stages (Table 6 and Figure 14).

Tableau 6: Place of collection, bioclimatic zone and extract yield of Tunisian spontaneous plants selected for the study

Sampl les	Tunisian spontaneous plants	Family	Place of collecti on	Biocli matic zone	Latitude	Longitud e	Plant crude extract yield (%)
E1	<i>L. guyonianum</i>	Plumbagin aceae	Gabes	LA	34.109491 N	9.982843 E	17,86
E2	<i>P. harmala</i>	Zygophylla cees	Sidi Bouzid	UA	35.035066 N	9.496925 E	18,14
E3	<i>R. chalepensis</i>	Rutaceae	zaghou ane	SH	36.388780 N	10.131152 E	14,57
E4	<i>R. communis</i>	Euphorbiac eae	zaghou ane	SH	36.387816 N	10.130768 E	12,71
E5	<i>N. retusa</i>	Nitrariacea e	Enfidha	LSA	36.079476 N	10.347530 E	15,00
E6	<i>C. cardunculus</i>	Asteracees	Enfidha	LSA	36.101872 N	10.388882 E	9,71
E7	<i>A. herba-alba</i>	Astéracees	Kairou an	UA	34.873042 N	10.082678 E	5,29
E8	<i>M. edule</i>	Aizoaceae	Ben arous	SH	36.715148 N	10.412255 E	29,71
E9	<i>T. gallica</i>	<i>Tamaricac eae</i>	Kairou an,	UA	35.867603 N	10.207805 E	17,00
E10	<i>D. stramonium</i>	Solanaceae	Ben arous	SH	36.712268 N	10.436624 E	21,00

SH: sub-humid; LSA: Lower semi-arid; UA: upper arid; La: Lower arid



Figure 14 : *C. cardunculus* collected at vegetative stage in its natural environment of the Enfidha region located in Tunisia which showed the best herbicidal activity.

The identification of the harvested plants was confirmed by Pr. Abderrazak Smaoui affiliated to the Biotechnology Center of Borj-Cédria (CBBC). All the selected plants were shade-dried for 15 days at 30°C. Seeds of *Phalaris minor* (*P. minor*) were collected in Tunisia from wheat fields. Seeds of *Trifolium incarnatum* (*T. incarnatum*), and *Sylibum marianum* (*S. marianum*) were obtained from ECOSEM Co. (Belgium).

Both extraction and fractionation of plant materials were carried out according to the optimised method of Falleh et al. (2013). Phenolic extracts were obtained by stirring 10 g of dry powder with 100 ml of methanol (Emplura EMD Millipore Corporation, a subsidiary of Merck KGaA) for 30 min. Methanol was eliminated using a rotavapor in vacuum at 45°C, and residues were re-dissolved in Tween 1%. Extracts were then kept at 4 °C for 24 h, filtered through a Whatman No 4 filter paper and stored at 4 °C until analysis. The extraction yields varied between 5.29 and 29.71 %.

Methanol filtrates were combined, concentrated under vacuum, and fractionated using a reverse-phase silica gel (Sigma-Aldrich, a subsidiary of Merck KGaA) to remove sugars and other polar compounds. The samples were loaded on a column containing 10 g of C18 resin per gram of dry extract, followed by 80 ml of water. The phenolic fractions adsorbed onto the resin were then eluted with 30 ml of increasing MeOH percentages (successively 20, 40, 60, 80 and 100% methanol). The fractions were evaporated separately.

2.2. Formulation

A formulation was designed to mix the plant extracts with vegetable oil so as to render the penetration of active molecules through epicuticular waxes easier. It contained amphiphilic substances in order to mix together a hydrophobic vegetable oil (for the product to stick to the leaves) and our hydrophilic extract. The formulations varied among the phenolic extracts and fractions; they are presented in Table 7.

Tableau 7: Composition of formulated herbicides

Composition	Extract 6 (%)	Fraction 2 (%)
Plant extract	3.40	-
Plant fraction	-	2.00
Vegetable oil of hazelnut	3.40	3.40
Ethoxylated (9) Castor oil ¹	0.67	0.67
Polyethylene glycol sorbitan monolaurate ²	0.33	0.33
UEP-100	0.25	0.25
Ethanol	0.50	0.50
Water	91.45	92.85
Total	100.00	100.00

¹CO₉ ²Tween 20

2.3. HPLC analysis

An HPLC system was used to identify the phenolic compounds. It was composed of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 400 bar (Agilent 1260, Agilent technologies, Germany) provided with a reverse-phase C18 analytical column of 4.6 x 100 mm and 3.5 µm particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200-400 nm. The column temperature was fixed at 25°C. Mobile phase B contained milli-Q water with 0.1% formic acid, whereas mobile phase A consisted of 0.2% methanol. The flow rate was kept at 0.4 ml/min. The optimised elution gradient was as follows: 0-5 min, 10-20% A; 5-10 min, 20-30% A; 10-15 min, 30-50% A; 15-20 min, 50-70% A; 20-25 min, 70-90% A; 25-30 min, 90-50% A; 30-35 min, return to initial conditions. A 2-µl volume was injected for each sample, and peaks were monitored at 280 nm. Phenolic compounds were identified based on retention times and the UV spectra of the phenolics chromatogram. The pure standards were myricitrin, quercetin, *p*-coumaric acid, naringenin and syringic acid. Identification was performed by comparing the retention times of the standards with those of the extracts. For the quantitative analysis, a calibration curve was obtained by plotting the peak areas against different concentrations for each identified compound at 280 nm: all the curves showed a strong linearity (with an average $r^2 = 0.99$): $y = 38,976x + 4,1296$ for syringic acid; $y = 32,266x + 17,439$ for *p*-coumaric acid; $y = 6,7915x - 35,235$ for myricitrin; $y = 9,5824x - 7,4659$ for quercetin, and $y = 23,691x - 88,898$ for naringenin. The amount of each compound was expressed in milligrams per gram of residue.

2.4. Pre-emergence activity under laboratory conditions

Seeds of *T. incarnatum*, *S. marianum* and *P. minor* were sterilised using 0.5 % sodium hypochlorite for 2 min. Each crude plant extract was first solubilised in Tween 1% and then diluted with distilled water to the desired concentration. Filter paper was moistened with 2 ml of Tween 1 % solution (which did not interfere with the assays) as a control, or with the crude methanolic extract solution prepared from different Tunisian plants at 5 g/L for the treatments. Ten seeds of *T. incarnatum*, *S. marianum* or *P. minor* were then immediately placed in Petri dishes, and three replicates (3 Petri dishes) were prepared for each extract and for each plant species. All Petri dishes were randomly placed in a growth chamber at $23 \pm 1^\circ\text{C}$, in darkness. The number of germinated seedlings was counted, and their hypocotyl and root lengths were measured

after 7 days. The inhibition rate of the root and hypocotyl lengths was calculated based on Equation (1):

$$\text{Inhibition rate (\%)} = T/C * 100, \quad (1)$$

Where T is the length of the roots or hypocotyls of the treated seedlings, and C represents the length of the roots or hypocotyls of the control seedlings.

2.5. Post-emergence activity under greenhouse conditions

A first post-emergence experiment was conducted to study the effects of the methanolic extracts on 2-to-3-week-old plantlets of *T. incarnatum*, *S. marianum*, and *P. minor* under controlled conditions. Seeds of *P. minor* were sown in boxes, whereas seeds of *T. incarnatum* and *S. marianum* were sown in pots. The plants were watered daily. When the weeds reached the 2-to-3-leaf stage, they were sprayed with 10 ml of one of the following solutions: a methanolic plant extract at 7.5, 20 or 34 g/L, or formulated plant extracts, or formulated fractions, or adjuvants only (as controls), or distilled water, or finally a commercial biological herbicide containing 34 g/L of pelargonic acid (as a positive control).

A second experiment was carried out on the herbicidal activity of the pure phenolic compounds identified in the most active fraction. Myricitrin, quercetin, naringenin are commercially available (Sigma, Belgium) and were sprayed only on *T. incarnatum* leaves for its strong resistance to pesticides (Mccurdy et al., 2013).

Whatever the post-emergence experiment, three replications were conducted for each treatment in a completely randomised manner. Seven days after spraying, the treated weed plants were examined to study wilting, necrosis, and chlorosis. The efficacy percentage was calculated using Equation (2):

$$\text{Percentage of efficacy (\%)} = N/T * 100, \quad (2)$$

Where N refers to the number of necrotic or withered leaves, and T represents the total number of leaves.

2.6. Statistical analysis

The results were analysed using Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA), using one-way analysis of variance (ANOVA) followed by Tukey's multiple range tests. The differences between individual means were considered significant only if $p < 0.05$. Therefore, values in a column followed by the same letter are not significantly different at $p < 0.05$.

3. Results

3.1. Effect of the plant extracts on weed germination and seedling growth

The phytotoxic effect of the different crude methanolic extracts obtained from aerial parts of Tunisian spontaneous plants is summarised in Figures 15 (germination) and 3 (seedling growth). The allelopathic influence on *T. incarnatum*, *S. marianum* and *P. minor* at 5 g/L on germination and seedling growth varied significantly depending on the plant extracts. A significant inhibitory effect on *T. incarnatum* seed germination was found only with the *Cynara cardunculus* (*C. cardunculus*) extract. In addition, all plant extracts significantly inhibited *S. marianum* germination as compared to the control. However, *C. cardunculus*, *Artemisia herba-alba* (*A. herba-alba*) and *Tamarix gallica* (*T. gallica*) best inhibited germination. *P. minor* was the most sensitive weed whatever the plant extract tested.

Concerning the inhibition of root and hypocotyl growth, the *C. cardunculus* crude methanolic extract had the highest inhibitory effect on *T. incarnatum* (Figure 16), which reached 87.57% for hypocotyl length. Altogether, the plant extracts had a higher inhibitory effect on hypocotyl rather than radicle growth. Moreover, the statistical analysis showed that the *C. cardunculus* and *A. herba-alba* plant extracts had the highest phytotoxic effect on *S. marianum* seedling growth, ranging between 80.87 and 100 %.

The phytotoxic effect of the plant extracts was higher on *P. minor* than on *T. incarnatum* and *S. marianum*. In fact, the majority of plant extracts including *C. cardunculus* and *A. herba-alba* completely inhibited *P. minor* seedling growth.

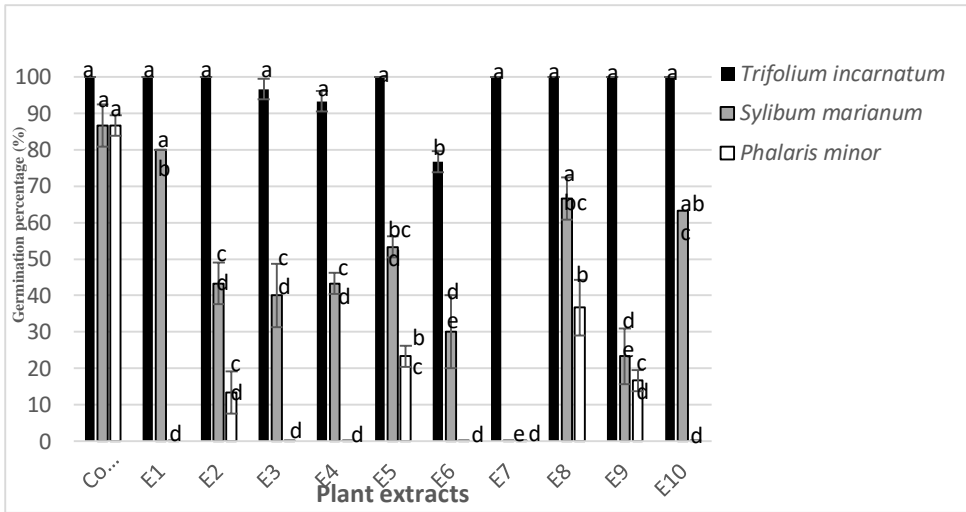


Figure 15 : Seed germination inhibition of weeds exposed for 7 days to 5g/L of different crude extracts obtained from the aerial parts of Tunisian spontaneous plants at the vegetative stage (E1: *L. guyonianum*; E2: *P. harmala*; E3: *R. chalepensis*; E4: *R. communis*; E5: *N. retusa*; E6: *C. cardunculus*; E7: *A. herba-alba*; E8: *M. edule*; E9: *T. gallica* E10: *D. stramonium*). For each weed, value in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

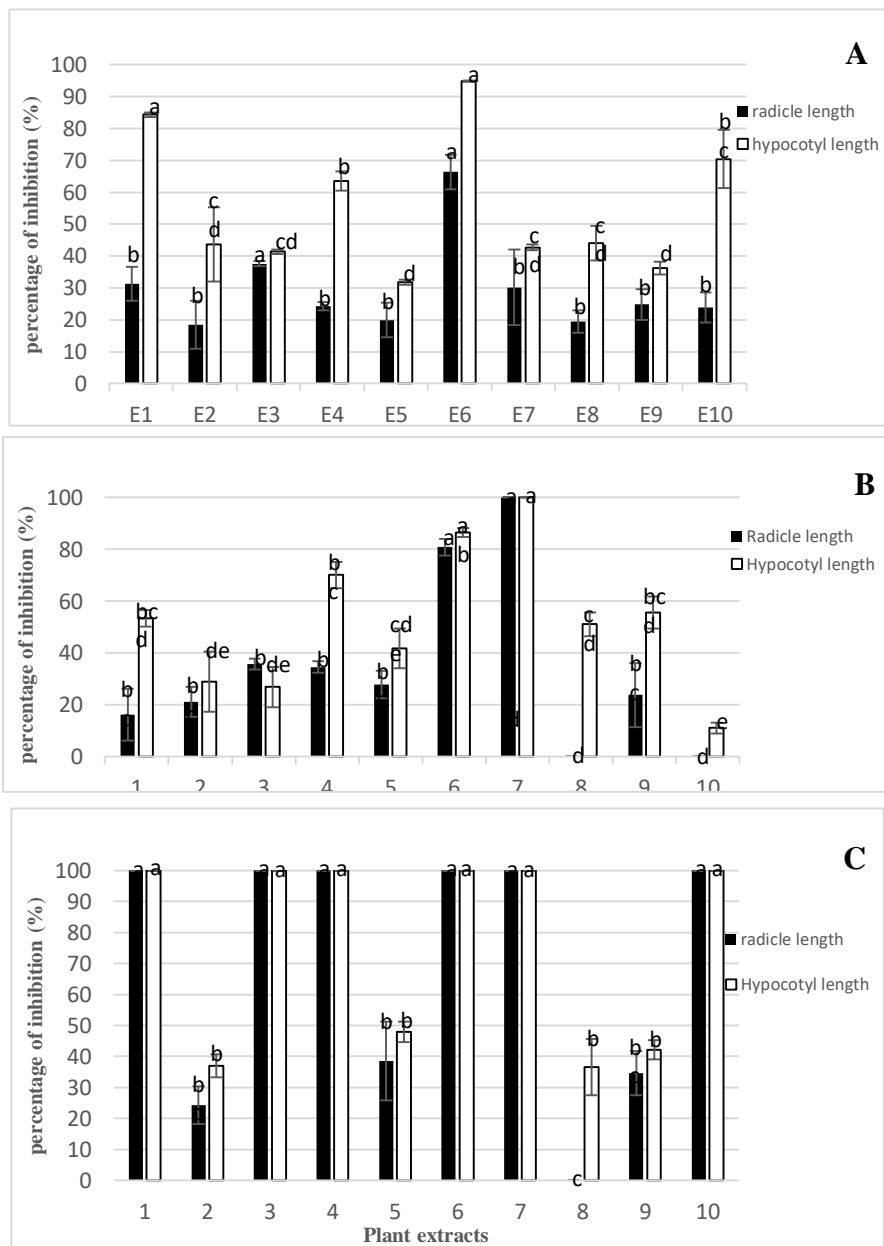


Figure 16: Phytotoxic effect of different crude extracts obtained from of aerial parts of Tunisian spontaneous plants at the vegetative stage at 5 g/L (E1: *L. guyonianum*; E2: *P. harmala*; E3: *R. chalepensis*; E4 *R. communis*; E5: *N. retusa*; E6: *C. cardunculus*; E7: *A. herba-alba*; E8: *M. edule*; E9: *T.gallica* E10: *D. stramonium*) on *T. incanatum* (A), *S. marianum* (B) and *P. minor* (C) seedling growth. For each part (radicle or hypocotyl) of each weed, value in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

3.2. Effect of the plant extracts on post-emerged weeds under greenhouse conditions

Different methanolic extracts were sprayed on *T. incarnatum*, *S. marianum*, and *P. minor* at 7.5, 20, or 34 g/L (Table 8). Only the *C. cardunculus* extract at 7.5 g/L and 20 g/L caused some necrosis and chlorosis on the leaves of all three weeds. Its herbicidal activity reached up to 37 %. At 34 g/L, several plant extracts had a phytotoxic effect against these weeds. *C. cardunculus* seemed to have again the best herbicidal activity, which reached 62.76%. That was why it was selected for the identification of bioactive compounds and the formulation of crude extracts and of the active fraction.

Tableau 8: Post-emergence activity of crude different extracts obtained from aerial parts of Tunisian spontaneous plants at the vegetative stage on *T. incarnatum*, *S. marianum* and *P. minor*.

Weeds	<i>T. incarnatum</i>			<i>S. marianum</i>			<i>P. minor</i>		
	7,5	20	34	7,5	20	34	7,5	20	34
Concentrations (g/L)									
<i>L. guyonianum</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	0.0± 0.0 ^D	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
<i>P. harmala</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	0.0± 0.0 ^D	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
<i>R. chalepensis</i>	0.0± 0.0 ^B	26.67 ± 2.22 ^B	30.0 ± 3.33 ^C	0.0 ±	0.0 ±	16.67± 4.44 ^B	0.0 ±	0.0± 0.0 ^B	12.33± 1.78 ^C
<i>R. communis</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	32.67 ± 1.78 ^C	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
<i>N. retusa</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	32.67 ± 5.11 ^C	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
<i>C. cardunculus</i>	24.33 ± 3.77 ^A	37.0 ± 2.0 ^A	62.67 ± 4.88 ^B	0.0 ±	17.0 ±	17.67± 1.78 ^B	17.0 ±	17.33 ±	16± 2.66 ^B
<i>A. herba-alba</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	29.33 ± 2.88 ^C	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
<i>M. edule</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	29.33 ± 3.78 ^C	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	17.67± 1.78 ^B
<i>T. gallica</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	28.33 ± 4.44 ^C	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
<i>D. stramonium</i>	0.0± 0.0 ^B	0.0± 0.0 ^C	31.33 ± 2.44 ^C	0.0 ±	0.0 ±	0.0± 0.0 ^C	0.0 ±	0.0± 0.0 ^B	0.0± 0.0 ^D
Biological herbicide¹	-	-	100.0 ± 0.0 ^A	-	-	100.0± 0.0 ^A	-	-	100.0± 0.0 ^A

¹ Natural herbicide based on pelargonic acid was used as a positive control (at the same concentration in the market). ² replicates were performed for each treatment. Value in a column followed by the same letter are not significantly different at P < 0.05, as established by Tukey's test.

3.3. Effect of *C. cardunculus* fractions on seed germination and seedling growth

The *C. cardunculus* crude methanolic extract was the most phytotoxic one among all the extracts tested. For this reason, a bioguided fractionation of the *C. cardunculus* crude methanolic extract was performed to identify the bioactive compounds that inhibited germination and seedling growth. Five fractions (named F1 to F5) were obtained and tested on *T. incarnatum*. The extract was chromatographed on silica gel, and the fractions were tested on *T. incarnatum* germination and seedling growth at 6 g/L. F1 and F2 seemed to be phytotoxic fractions in terms of inhibition of germination (Figures 17, 18), i.e., 30% and 60% inhibition after 5 days, respectively. However, the statistical analysis showed that the germination rate of *T. incarnatum* treated with F3, F4, and F5 was similar to that of the untreated seeds. F1 and F2 nearly completely inhibited *T. incarnatum* seedling growth (Figure 17). Conversely, the two less polar fractions (F4 and F5) did not have any significant effect on *T. incarnatum* seedling growth, while F3 had an intermediary effect.

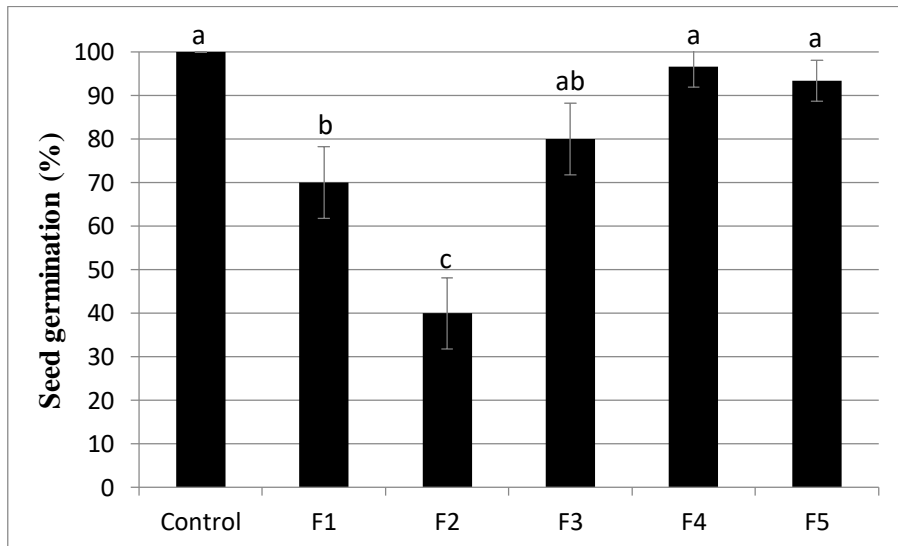


Figure 17 : Percentage of *T. incarnatum* germination after 5 days treated by one of the following fractions: F1: fraction 1 (20 % methanol); F2: fraction 2 (40% methanol); F3: fraction 3 (60% methanol); F4: fraction 4 (80% methanol); F5: fraction 5 (100 % methanol). Fractions were obtained by fractionation of *C. cardunculus*

methanolic crude extract. Value in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

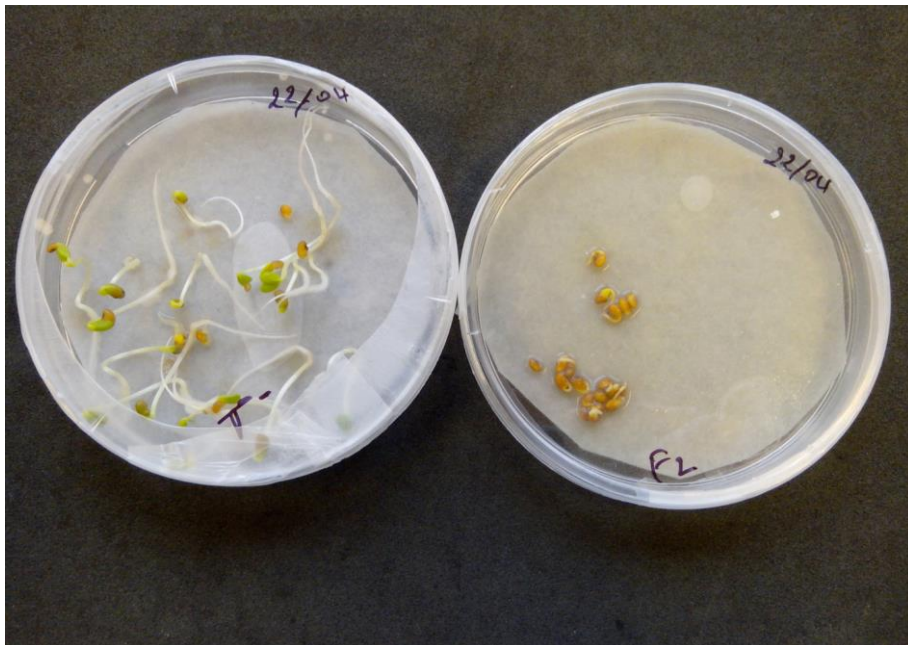


Figure 18 : Inhibitory effects of *C. cardunculus* plant extract, obtained from aerial plant at vegetative stage, on the germination and seedling growth of *T. incarnatum* at 6 g/L after 5 days. Left: Untreated *T. incarnatum* Right: Treated *T. incarnatum* with *C. cardunculus* plant fraction at 6 g/L after 5 days.

3.4. Phytochemical investigation of the *C. cardunculus* crude methanolic extract and its fractions

HPLC analysis was carried out to identify the phenolic compounds of the *C. cardunculus* crude methanolic extract and its fractions. The chemical profile showed the presence of 5 phenolic compounds in common in the first polar fractions (F1, F2, and F3). These compounds were syringic acid, *p*-coumaric acid, myricitrin, quercetin and naringenin (Table 9). No phenolic compound was identified in fractions 4 and 5, which had no effect on *T. incarnatum* germination or seedling growth. The amount of these compounds was low in the crude *C. cardunculus* extract, with 0.108, 0.487, 0.755, 0.383, and 0.359 mg/g dry weight (DW), respectively, as compared to the fractions.

p-coumaric acid, quercetin and myricitrin were concentrated in F1, F2 and F3 (41.209, 17.427, and 64.764 mg/g DW, respectively).

Tableau 9: Phenolic compounds identified and quantified in *C. cardunculus* crude methanolic extract and its obtained fractions.

Identified phenolic compounds	Retention time (min)	Molecular formula	Concentrations in mg/ g of DW					
			Crude extract	F1 ¹	F2	F3	F4	F5
Syringic acid	17.8	C ₉ H ₁₀ O ₅	0,108	NI	NI	1,308	NI	NI
Myricitrin	20.7	C ₂₁ H ₂₀ O ₁₂	0,755	NI	3,088	64,764	NI	NI
<i>p</i> -coumaric acid	20.09	C ₉ H ₈ O ₃	0,487	41,209	NI	0,576	NI	NI
Quercetin	23.7	C ₁₅ H ₁₀ O ₇	0,383	NI	17,427	1,430	NI	NI
Naringenin	24.3	C ₁₅ H ₁₂ O ₅	0,359	NI	4,486	NI	NI	NI

NI: not identified %

¹F1: fraction 1 (20 % methanol); F2: fraction 2 (40% methanol); F3: fraction 3 (60% methanol); F4: fraction 4 (80% methanol); F5: fraction 5 (100 % methanol).

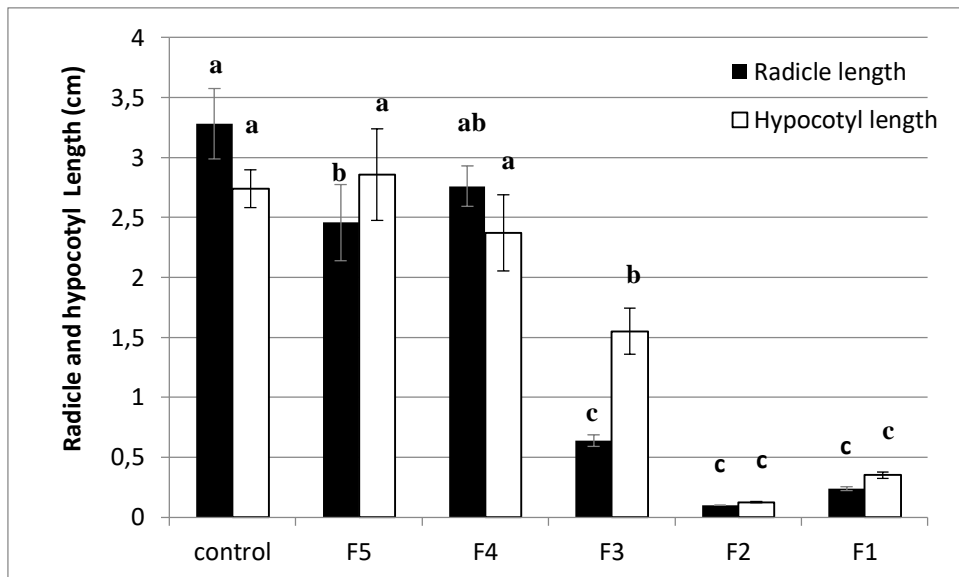


Figure 19: Radicle and hypocotyl length of *T. incarnatum* after 5 days treated by 5 fractions F1: fraction 1 (20 % methanol); F2: fraction 2 (40% methanol); F3: fraction 3 (60% methanol); F4: fraction 4 (80% methanol); F5: fraction 5 (100 % methanol) obtained by fractionation of *C. cardunculus* methanolic crude extract. C. Value in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

3.5. Effect of the formulated crude *C. cardunculus* extract and active fraction on post-emerged weeds under greenhouse conditions

The spraying of formulated *C. cardunculus* crude methanolic extract burnt *T. incarnatum* down. Six hours after spraying, *T. incarnatum* leaves wilted rapidly, followed by the stems 3 days later, demonstrating that the formulation allowed active compounds to pass into the stems. Fraction 2 from the *C. cardunculus* crude methanolic extract was also selected for formulating because it was the most efficient one on pre-emergence activity. At 20 g/L, it had the same effect on *T. incarnatum* as pelargonic acid at 34 g/L (Figure 20). It caused total leaf drying followed by plant death, as shown in Figure 21.

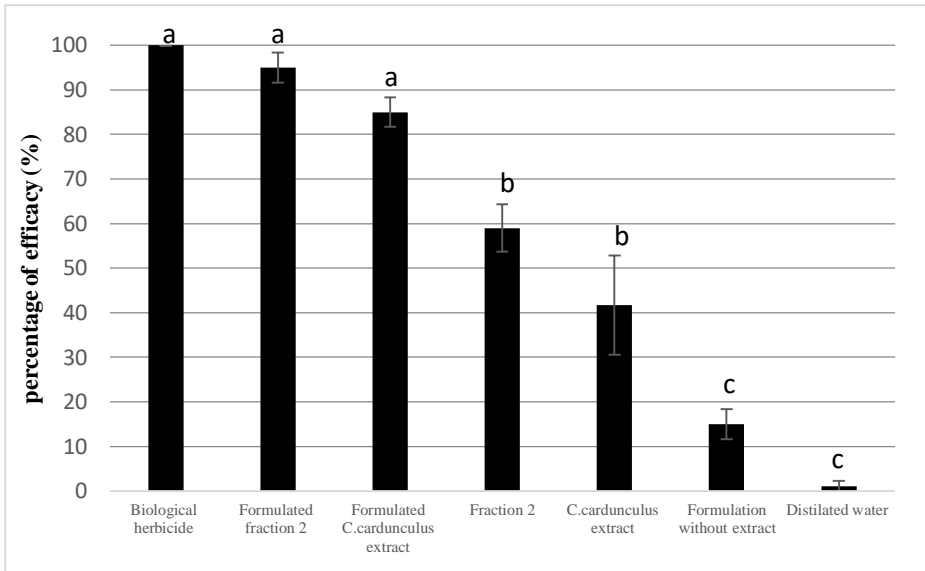


Figure 20 : Post-emergence activity of different herbicide compositions on *T. incarnatum* after 5 days of treatment: *C. cardunculus* crude methanolic extract with and without formulation at 34 g/L; fraction 2 with and without formulation at 20 g/L isolated from methanolic crude extract of *C. cardunculus* plant extract; biological herbicide based on pelargonic acid at 34 g/L (the same concentration as in the market), formulation contained only vegetable oil and adjuvants. Value in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.



Figure 21 : Herbicidal activity of formulated fraction obtained from *C. cardunculus* plant extract by fractionation on *T. incarnatum* after 7 days. Left : Treated *T. incarnatum* with formulated *C. cardunculus* plant fraction at 20 g/L. Right : Treated *T. incarnatum* without plant extract.

3.6 Effect of the identified phenolic compounds on post-emerged weeds under greenhouse conditions

In that last trial, quercetin, naringenin or myricitrin, which had been identified in the most active fraction, were sprayed on *T. incarnatum* at 250 $\mu\text{g/ml}$, 90 $\mu\text{g/ml}$ and 60 $\mu\text{g/ml}$, respectively, i.e., the concentrations recorded in the most active fraction (F2) when used at 20 g/L. These compounds (formulated like F2) had a significant phytotoxic effect as compared to the compound-free formulation (Figure 22). Efficacy reached 52.33% with formulated myricitrin.

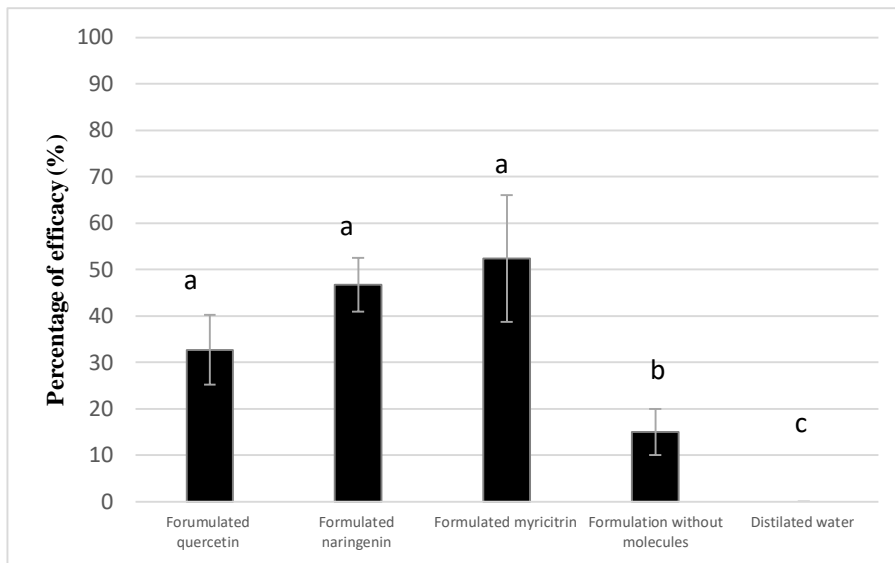


Figure 22 : Post-emergence activity of formulated phenolic compounds on *T. incarnatum* after 5 days of treatment: quercetin, naringenin, myricitrin were sprayed respectively at 250 $\mu\text{g/ml}$, 90 $\mu\text{g/ml}$ and 60 $\mu\text{g/ml}$ which are the concentrations in the most active fraction (F2) at 20 g/L. The formulation contained vegetable oil and adjuvants. Value in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

4. Discussion

We determined the herbicidal activity of ten Tunisian spontaneous plants in pre- and post-emergence to select the most promising plant(s) for potential use as a bioherbicide. The greatest herbicidal activity was obtained using extracts from two *Asteraceae* plants (*A. herba-alba* and *C. cardunculus*), which inhibited seed germination and seedling growth of three weeds. The *Asteraceae* family has been found to harbour the most prominent biocidal substances for agriculture and to be a good source for isolating and purifying allelopathic secondary metabolites (Bessada et al., 2015; Watanabe et al., 2014). In addition, the *C. cardunculus* crude methanolic extract had the best herbicidal activity in post-emergence under greenhouse conditions. *C. cardunculus* leaves are known to be a good source of polyphenols (Omezzine et al., 2011; Falleh et al., 2008) and could inhibit the development of other invasive weeds such as barnyard grass (*Echinochloa crus-galli*) and

Brachiaria sp. (Rial et al., 2014). The herbicidal activity of phenolic extracts in pre-emergence is widely documented (Javaid et al., 2010; Araniti et al., 2015; Watanabe et al., 2014; Nekonam et al., 2014; Bessada et al., 2015; Uddin et al., 2014; Ma et al., 2018; Schleiden et al., 2019), but to our knowledge no study had yet focused on their effect in post-emergence by direct spraying on weed leaves. *C. cardunculus*, which is well known for its coagulant properties (Prados & Pino, 2007) and phytotoxic effect in pre-emergence (Rial et al., 2014; Rial et al., 2016; Scavo et al., 2019), is unveiled for the first time for its herbicidal effect in post-emergence. A formulation was used to improve its efficiency, containing a vegetable oil and nonionic surfactants. The latter can increase the adsorption rate of polar molecules, dissolve cuticular fatty acids and therefore improve the penetration of the hydrophilic active substance (Batish et al., 2007; Hazrati et al., 2017). Based on these data, a recent study showed that a formulation based on palm oil, Tween 20 and Span 80 improved the herbicidal activity of *Phoma* sp. metabolites (Toderò et al., 2018).

Many authors have demonstrated that the phytotoxic effect of plant extracts was related to the presence of phenolic compounds (Li et al., 2010; Lim et al., 2017; Yan et al., 2014; Ribeiro et al., 2015; Araniti et al., 2014; Jelassi et al., 2016; Ben El Hadj Ali et al., 2014; Araniti et al., 2015; Sbai et al., 2016; Javaid et al., 2010; Nebo et al., 2014). Methanol seems to be the best solvent to extract phenolics due to their good solubility in it (Ben El Hadj Ali et al., 2014). In this context, our study showed that the most bioactive fraction isolated by HPLC analysis from the *C. cardunculus* crude methanolic extract contained three flavonoids: myricitrin, naringenin and quercetin. Each of these flavonoids had a significant phytotoxic effect on *T. incarnatum*. In this context, De Martino et al. (2012) reported a significant phytotoxic effect of quercetin and naringenin on radish. To our knowledge, myricitrin was tested for the first time in our experiments. In the same line, Javaid et al. (2010) isolated flavonoids from mango (*Mangifera indica* L.) and showed that they caused yellowing of parthenium (*Parthenium hysterophorus* L.) seedlings. Moreover, flavonoids purified from a root extract of *Stellera chamaejasme* L. collected in China acted as potential major phytotoxins against *Arabidopsis thaliana* (Yan et al., 2014). Finally, fractionation of *Derris urucu* ethanol extracts permitted the identification of three new dihydroflavonols named

urucuol A, urucuol B and isotirumalin, which showed potential herbicidal activity (Da Silva et al., 2013).

The mode of action of allelochemicals, including the flavonoids highlighted in our study, still remains unknown (Soltys et al., 2013; Vyvyan, 2002). In fact, these secondary metabolites act as toxins for the plant metabolism and affect several physiological functions of plant cells such as membrane integrity, photosynthesis and respiration, metabolic and proteomic activity, phytohormonal activity, and ion uptake (Yan et al., 2014; Ribeiro et al., 2015; Yan et al., 2015; Ahmed et al., 2017). They modify the expression of one or more genes, which then leads to plant death (Cordeau et al., 2016). Chalcone, an aromatic precursor of the synthesis of these flavonoids, induced programmed cell death in *Arabidopsis thaliana* roots (Tielas et al., 2013). In the same line, phenolic acids act as phytotoxic agents by inducing overproduction of reactive oxygen species (ROS) that disturb respiration and photosynthesis (Lim et al., 2017; Ladhari et al., 2014; Franco et al., 2015; Araniti et al., 2014). These ROS play an important signalling role in the control of a number of essential processes like growth, development, the response to environmental constraints, pathogen defence and stomatal behaviour. They can generate mutations, react with DNA, proteins, and lipids, inducing tissue injury, membrane damage, and programmed cell death (PCD) processes (Yan et al., 2015). Flavonoids are known for their antioxidant properties (de Martino et al., 2012; Belmekki & Bendimerad, 2012; Sakihama et al., 2002), but under certain conditions (concentration, pH modification, solubility characteristics, potential metal-reducing chelating behaviour), they have potential prooxidant properties (Eghbaliferiz & Iranshahi, 2016). The phenolic compounds such as myricitrin, naringenin and quercetin – identified in our active fraction – are involved in diverse physiological effects such as mineral uptake alteration, disruption of membrane permeability, stomatal closure, induction of water stress, deleterious effects on photosynthesis and protein synthesis, and alteration of enzyme activities. Flavonoids cause overproduction of phenoxyl radicals, directly linked to lipid peroxidation and ROS accumulation in the cell, and this can damage DNA, lipids, and other biological molecules (Sakihama et al., 2002; de Martino et al., 2012; Ribeiro et al., 2015). Moreover, on a morphological scale, we noticed that root inhibition in response to different methanolic plant extracts was related to the development of thin secondary roots. This can be caused by oxidative stress

(Franco et al., 2015), potentially due to the interaction of phenolic compounds with auxin, cytokinin, and gibberellin transport (Ribeiro et al., 2015). In accordance with our study, Franco et al. (2015) found that exogenous flavonoids, such as those identified in our *C. cardunculus* extract, could delay primary root growth and improve the expansion of lateral roots. They modified the expression model of specific genes involved in root tissue differentiation.

5. Conclusions

Our results show that *C. cardunculus* is a promising plant with pre-emergence but also post-emergence herbicidal activity under greenhouse conditions. Its formulated active fraction at 20 g/L had the same herbicidal effect as commercial pelargonic acid at 34 g/L. This opens new perspectives on the application of Tunisian plant extracts as novel botanical herbicides for weed management. Finally, myricitrin was identified for the first time as one of the bioactive molecules contained in the active fraction. It can be employed in a new herbicidal formulation and offers new strategies and pathways for the biopesticide industry to create an eco-friendly alternative to chemical herbicides. To go one step further, it could be really interesting to determine the mode(s) of action of the *C. cardunculus* crude methanolic extract and of its active compounds such as myricitrin on weeds and to optimise the formulation to improve the effectiveness and stability of the emulsion.

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Conflict of interest

The authors declare no conflict of interest.

References

- Ahmed, H.M., 2017. Natural Product Research Formerly Natural Product Letters Phytochemical screening, total phenolic content and phytotoxic activity of corn (*Zea mays*) extracts against some indicator species. *Nat. Prod. Res.* 6419.
- Araniti, F., Mancuso, R., Lupini, A., Giofrè, S., Sunseri, F., Gabriele, B., Abenavoli, M., 2015. Phytotoxic Potential and Biological Activity of Three Synthetic Coumarin Derivatives as New Natural-Like Herbicides. *Molecules* 20, 17883–17902.
- Araniti, F., Sunseri, F., Abenavoli, M.R., 2014. Phytotoxic activity and phytochemical characterization of *Lotus ornithopodioides* L., a spontaneous species of Mediterranean area. *Phytochem. Lett.* 8, 179–183.
- Batish, D.R., Singh, H.P., Setia, N., Kohli, R.K., Kaur, S., Yadav, S.S., 2007. Alternative control of littleseed canary grass using eucalypt oil. *Agron. Sustain. Dev.* 27, 171–177.
- Ben El Hadj Ali, I., Bahri, R., Chaouachi, M., Boussai'd, M., Harzallah-Skhiri, F., 2014. Phenolic content, antioxidant and allelopathic activities of various extracts of *Thymus numidicus* Poir. organs. *Ind. Crops Prod.* 62, 188–195.
- Ben Ghnaya, A., Hanana, M., Amri, I., Balti, H., Gargouri, S., Jamoussi, B., Hamrouni, L., 2013. Chemical composition of *Eucalyptus erythrocorys* essential oils and evaluation of their herbicidal and antifungal activities. *J. Pest Sci.* 86, 571–577.
- Bessada, S.M.F., Barreira, J.C.M., Oliveira, M.B.P.P., 2015. Asteraceae species with most prominent bioactivity and their potential applications: A review. *Ind. Crops Prod.* 76, 604–615.
- Bouabidi, W., Hanana, M., Gargouri, S., Amri, I., Fezzani, T., Ksontini, M., Jamoussi, B., Hamrouni, L., 2015. Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis* L. essential oils. *Nat. Prod. Res.* 29, 864–8.

- Chengxu, W., Mingxing, Z., Xuhui, C., Bo, Q., 2011. Review on Allelopathy of Exotic Invasive Plants. *Procedia Eng.* 18, 240–246.
- Da Silva, E.A.S., Lôbo, L.T., Da Silva, G.A., Souza Filho, A.P. da S., Da Silva, M.N., Arruda, A.C., Guilhon, G.M.S.P., Santos, L.S., Arruda, M.S.P., 2013. Flavonoids from leaves of *Derris urucu*: Assessment of potential effects on seed germination and development of weeds. *An. Acad. Bras. Cienc.* 85, 881–889.
- Dayan, F.E., Owens, D.K., Duke, S.O., 2012. Rationale for a natural products approach to herbicide discovery. *Pest Manag. Sci.* 68, 519–528.
- Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., Abdelly, C., 2008. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Comptes Rendus - Biol.* 331, 372–379.
- Falleh, H., Msilini, N., Oueslati, S., Ksouri, R., Magne, C., Lachaâl, M., Karray-Bouraoui, N., 2013. *Diplotaxis harra* and *Diplotaxis simplex* organs: Assessment of phenolics and biological activities before and after fractionation. *Ind. Crops Prod.* 45, 141–147.
- Flamini, G., 2012. Natural herbicides as a safer and more environmentally friendly approach to weed control: A review of the literature since 2000, 1st ed, *Studies in Natural Products Chemistry*.
- Franco, D.M., Silva, E.M., Saldanha, L.L., Adachi, S.A., Schley, T.R., Rodrigues, T.M., Dokkedal, A.L., Nogueira, F.T.S., Rolim de Almeida, L.F., 2015. Flavonoids modify root growth and modulate expression of SHORT-ROOT and HD-ZIP III. *J. Plant Physiol.* 188, 89–95.
- Hazrati, H., Saharkhiz, M.J., Niakousari, M., Moein, M., 2017. Natural herbicide activity of *Satureja hortensis* L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. *Ecotoxicol. Environ. Saf.* 142, 423–430.
- Heleno, S.A., Martins, A., João, M., Queiroz, R.P., Ferreira, I.C.F.R., 2015. Bioactivity of phenolic acids : Metabolites versus parent compounds : A review. *FOOD Chem.* 173, 501–513.
- Jabran, K., Mahajan, G., Sardana, V., Chauhan, B.S., 2015. Allelopathy for

weed control in agricultural systems. *Crop Prot.* 72, 57–65.

- Javaid, A., Shafique, S., Kanwal, Q., 2010. Herbicidal activity of flavonoids of mango leaves against *Parthenium hysterophorus* L. *Nat. Prod. Res.* 24, 1865–1875.
- Jelassi, A., Ayeb-Zakhama, A. El, Nejma, A. Ben, Chaari, A., Harzallah-Skhiri, F., Jannet, H. Ben, 2016a. Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species. *Ind. Crops Prod.* 83, 339–345.
- Ksouri, R., Ksouri, W.M., Jallali, I., Debez, A., Magné, C., Hiroko, I., Abdelly, C., 2012. Medicinal halophytes : potent source of health promoting biomolecules with medical , nutraceutical and food applications. *Crit. Rev. Biotechnol.* 32, 289-326.
- Ladhari, A., Omezzine, F., Haouala, R., 2014. The impact of Tunisian Cappariaceae species on cytological, physiological and biochemical mechanisms in lettuce. *South African J. Bot.* 93, 222–230.
- Li, Z.-H., Wang, Q., Ruan, X., Pan, C.-D., Jiang, D.-A., 2010. Phenolics and Plant Allelopathy. *Molecules* 15, 8933–8952.
- Lim, C.J., Basri, M., Ee, G.C.L., Omar, D., 2017. Phytoinhibitory activities and extraction optimization of potent invasive plants as eco-friendly weed suppressant against *Echinochloa colona* (L.) Link. *Ind. Crops Prod.* 100, 19–34.
- Ma, S., Fu, L., He, S., Lu, X., Wu, Y., Ma, Z., Zhang, X., 2018. Potent herbicidal activity of *Sapindus mukorossi* Gaertn. against *Avena fatua* L. and *Amaranthus retroflexus* L. *Ind. Crops Prod.* 122, 1–6.
- Morra, M.J., Popova, I.E., Boydston, R.A., 2018. Bioherbicidal activity of *Sinapis alba* seed meal extracts. *Ind. Crops Prod.* 115, 174–181.
- Nebo, L., Varela, R.M., Molinillo, J.M.G., Sampaio, O.M., Severino, V.G.P., Casal, C.M., Fernandes, M.F.D.G., Fernandes, J.B., Macías, F. a., 2014. Phytotoxicity of alkaloids, coumarins and flavonoids isolated from 11 species belonging to the Rutaceae and Meliaceae families. *Phytochem. Lett.* 8, 226–232.

- Nekonom, M.S., Razmjoo, J., Kraimmojeni, H., Sharifnabi, B., Amini, H., Bahrami, F., 2014. Assessment of some medicinal plants for their allelopathic potential against redroot pigweed (*Amaranthus retroflexus*) J. plant. prot. Res. 54.
- Omezzine, F., Ladhari, A., Rinez, A., Haouala, R., 2011. Potent herbicidal activity of *Inula crithmoides* L. Sci. Hortic. 130, 853–861.
- Ribeiro, R.C., Feitoza, R.B.B., Lima, H.R.P., Geraldo De Carvalho, M., 2015. Phytotoxic effects of phenolic compounds on *Calopogonium mucunoides* (Fabaceae) roots. Aust. J. Bot. 63, 679–686.
- Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H., 2002. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxicology. 177, 67–80.
- Sbai, H., Saad, I., Ghezal, N., Greca, M. Della, Haouala, R., 2016. Bioactive compounds isolated from *Petroselinum crispum* L. leaves using bioguided fractionation. Ind. Crops Prod. 89, 207–214.
- Soltys, D., Krasuska, U., Bogatek, R., Gniazdowska, a., 2013. Allelochemicals as Bioherbicides - Present and Perspectives. Herbic. - Curr. Res. Case Stud. 517–542.
- Suksungworn, R., Srisombat, N., Bapia, S., Soun-Udom, M., Sanevas, N., Wongkantrakorn, N., ... Duangsrissai, S. (2017). Coumarins from *Haldina cordifolia* lead to programmed cell death in giant mimosa: Potential bio-herbicides. Pakistan Journal of Botany, 49(3), 1173–1183
- Trabelsi, N., Oueslati, S., Henry-vitrac, C., Waffo-téguo, P., Medini, F., Mérillon, J., Abdelly, C., Ksouri, R., 2013. Phenolic contents and biological activities of *Limoniastrum guyonianum* fractions obtained by Centrifugal Partition Chromatography. Ind. Crop. Prod. 49, 740–746
- Uddin, M.R., Park, S.U., Dayan, F.E., Pyon, J.Y., 2014. Herbicidal activity of formulated sorgoleone, a natural product of sorghum root exudate. Pest Manag. Sci. 70, 252–7.
- Yan, Z., Guo, H., Yang, J., Liu, Q., Jin, H., Xu, R., Cui, H., Qin, B., 2014.

Phytochemistry Phytotoxic flavonoids from roots of *Stellera chamaejasme* L. (Thymelaeaceae). *Phytochemistry* 106, 61–68.

Yan, Z., Wang, D., Ding, L., Cui, H., Jin, H., Yang, X., Yang, J., Qin, B., 2015. Plant Physiology and Biochemistry Mechanism of artemisinin phytotoxicity action : Induction of reactive oxygen species and cell death in lettuce seedlings. *Plant Physiol. Biochem.* 88, 53–59.

Vyvyan, J.R., 2002. Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron* 58, 1631–1646.

Watanabe, Y., Novaes, P., Varela, R.M., Molinillo, J.M.G., Kato-Noguchi, H., Macias, F.A., 2014. Phytotoxic Potential of *Onopordum acanthium* L. (Asteraceae). *Chem. Biodivers.* 11, 1247–1255.

Study of *Rosmarinus officinalis* essential oil as an effective antifungal and herbicidal agent

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Abstract

In order to reduce the use of chemical pesticides, great interest has been focused on environment-friendly biological control agents and botanicals that preserve biodiversity. In this context, our study aimed to assess the antifungal and herbicidal activities of *Rosmarinus officinalis* essential oil (EO) to find an alternative to synthetic pesticides. The chemical composition of *Rosmarinus officinalis* essential oil was determined by gaz chromatography-mass spectrometry analysis (GC-MS). Results showed that *R. officinalis* essential oil was rich in monoterpenes and the major constituents were 1,8-cineole (54.6%), camphor (12.27%) and α -pinene (7.09%). However, under laboratory condition, two tests were carried out. The first one consisted on the study of EO antifungal activity using ELISA microplates and the second one consisted on evaluating the effect of EO on seedling growth of weeds. It was confirmed that this EO significantly inhibits spore germination of *Fusarium oxysporum*, *Fusarium culmorum*, *Penicillium italicum* and at 6 mM, the percentage of inhibition reached 100% on *Fusarium oxysporum*. Indeed, EO slows down seedling growth of *Trifolium incarnatum*, *Silybum marianum*, and *Phalaris minor*. In fact, EO at 5 mM completely inhibits seed germination. On the other hand, another experiment was carried out to evaluate the herbicidal activity by spraying EO on weeds. This showed that a novel herbicide formulation was set up for the first time to improve the activity of *R. officinalis* EO on post-emergence. Overall, *R. officinalis* EO could be suggested as a potential eco-friendly pesticide and suitable source of natural compounds potentially usable as natural pesticides.

Additional keywords: biological control; 1,8 cineole, fungicidal activity; bio-herbicidal activity; formulation.

Abbreviations used: EO (essential oil);

PDA (Potato dextrose agar)

PDB (Potato dextrose broth)

1. Introduction

Agricultural production has always been threatened by the presence of plant pathogens such as fungi, bacteria, and viruses (Usanmaz, Cakir, Komaki, & Ercisli, 2016). Moreover, weeds are another major issue. In fact, they compete with crops for resources (water, nutrients, light) and cause huge economic losses, up to 34% in major crops (Fabrizio Araniti et al., 2015). Every year, approximately 2.5 million tons of pesticides are used on crops worldwide to fight plant diseases (Koul *et al.*, 2008) with consequences on human health, soils and the environment (groundwater contamination and development of weed resistance). This intensive use has been recognized as one of the main drivers of biodiversity losses (Schütte *et al.*, 2017).

In the last few decades, there has been growing interest in investigating eco-friendly alternatives, in particular essential oil (EO)-based methods in order to curtail pesticide use because pesticides cause extensive damage to agricultural and natural systems (Ben Ghnaya *et al.*, 2013). Moreover, the use of EOs obtained through a cheap production process can reduce the frequent applications of certain synthetic pesticides that have deleterious effects on the environment and human health (Pavela & Benelli, 2016). But EOs can also present a low health risk during application. One of the great challenges for further research is to design an efficient stabilization process so as to apply EOs in fields. In the same vein, several studies have pointed out that EOs may have not only antifungal activity (Koul *et al.*, 2008; Tian *et al.*, 2012; Ahluwalia *et al.*, 2014; Hmiri *et al.*, 2015; Boubaker *et al.*, 2016) but also the ability to inhibit weed seedling growth (Uremis *et al.*, 2009; Poonpaiboonpipat *et al.*, 2013) In the Mediterranean region and especially in Tunisia, the most widespread botanical family is Lamiaceae, which has antimicrobial properties (Pintore *et al.*, 2002). Among these aromatic plants, the most interesting species is *Rosmarinus officinalis* (*R. officinalis*) which is known for its antifungal activity (Angioni *et al.*, 2004; Giamperi *et al.*, 2011; Hmiri *et al.*, 2015) and its richness in EOs characterized by the predominance of monoterpenes – mostly 1,8 cineole –, camphor, and α -pinene. Hence, the main aims of this study were (1) to assess the antifungal activity of *R. officinalis* EO against three potential plant-pathogenic fungi, (2) to evaluate its herbicidal activity on three weed species for the first time, and then (3) to formulate a bioherbicide in order to enhance its efficiency and stability.

2. Material and methods

2.1 Plant material and essential oil extraction

In March 2014, *R. officinalis* plants, which belong to the *Lamiaceae* family, were collected at the flowering stage in a naturally diversified mountain of the Seliana region in the northeast of Tunisia (36°06'47.9"N 9°35'30.0"E). The plants were identified by the botanist of the Biotechnology Center of Borj-Cedria (CBBC). All selected plants were shade-dried for 15 days at 30°C. One hundred grams of dried leaves and flowers were chopped and subjected to hydrodistillation using a Clevenger-type apparatus for 2 h (Ben Jemia *et al.*, 2015). The essential oil was stored at 4°C in amber vials.

2.2. GC–MS analysis

The EOs were analyzed by a gas chromatography-mass spectrometry analyzer (Hewlett Packard HP5890 series II, USA) equipped with an HP-5 column coated with 5% phenyl methyl siloxane (30 m × 250 µm × 0.25 µm). The carrier gas was helium, at a pressure of 1 ml/min. The mass spectrometer (Agilent Technologies, USA) ionized the compounds at an electron impact of 70 eV prior to identification. The program was the following one: 40 °C for 1 min, then a 4 °C/min increase up to 100 °C, 100 °C for 5 min, followed by a 6 °C/min increase up to 200 °C, then 200 °C for 5 min, and finally a 15 °C/min increase up to 250 °C. The total running time for each sample was 46 min. The components were identified by comparison with the W9N11.L library and calculated retention indexes relatively to C₈-C₂₄ n-alkanes injected in the HP 5MS column. The relative area percentages of the different EO constituents were calculated from the peak areas of the total ions.

2.3. Formulation

A formulation was used to mix the EOs in water and facilitate the penetration of active molecules through the epicuticular waxes. It contained amphiphilic substances to render interactions between polar and non-polar parts possible. The compounds of the formulation were chosen to allow for better stability, efficacy, and a small droplet size. The detailed composition of the formulation is presented in Table 10.

Tableau 10 : Composition of the formulated natural herbicide based on the use of *Rosmarinus officinalis* essential oil.

Composition	% Content
Essential oil	3.4
Hazelnut vegetable oil	3.4
Tween 20	0.7
Span 80	0.3
Aplus UEP-100	0.25
Ethanol	0.5
Water	91.45
Total	100

2.4. Evaluation of the antifungal activity

2.4.1 Preparation of the culture media

Potato dextrose agar (PDA) was used to grow the fungal pathogens in Petri dishes, while potato dextrose broth (PDB) and tomato juice (V8) were used for growth in ELISA microplates.

2.4.2 Fungal strains and preparation of the inoculum

The fungal species *Fusarium oxysporum* (*F. oxysporum*) (MUCL 38936), *Fusarium culmorum* (*F. culmorum*) (MUCL28166) and *Penicillium italicum* (*P. italicum*) (MUCL 15608) were obtained from the BCCM/MUCL Agro-food & Environmental Fungal Collection (Louvain La Neuve, Belgium). They were cultured on PDA and incubated at 20°C under a 16h L: 8h D photoperiod.

A spore suspension was made by adding 10 mL of sterile distilled water to 0.05% Tween 20 on the surface of a 14-day-old fungal colony. The surface was gently scratched to suspend the spores in the liquid. The spore suspension was filtered through a sterilized double layer of fine cloth to remove mycelial fragments. The spore concentration was adjusted to 10⁶ spores/mL with a Bürker haemocytometer.

The antifungal activity of the EO was evaluated using ELISA microplates with a randomized block design, as described by (Kaddes et al.,

2016)). The growth of each pathogen was monitored in a volume of 200 μL containing diluted (3.10^{-2} v/v) PDB medium for *P. italicum* and *F. oxysporum*, and V8 medium for *F. culmorum*, the inoculum, and the EO at 1, 3, and 6 mM. The optical density of each well was measured at a wavelength of 630 nm every 24 h for 120 h, using a spectrophotometer for ELISA plates. Eight replications were conducted for each concentration, and tween 20 at 1% v/v was used as a negative control. The inhibition percentages were then calculated using the following equation:

$$\begin{aligned} & \% \text{ inhibition} \\ & = \frac{AV(ODX'(t = 0) - ODX'(t = 120h)) - AV(ODHx(t = 0) - ODHx(t = 120h))}{AV(ODX'(t = 0) - ODX'(t = 120h))} \end{aligned}$$

where AV is the average value, $ODX'(t=0)$ is the optical density of the pathogen growth control just after inoculation, $ODX'(t=120)$ is the optical density of the pathogen growth control after 120 h, $ODHx(t=0)$ is the optical density of the pathogen in association with the EO just after inoculation, and $ODHx(t=120h)$ is the optical density of the pathogen in association with the EO after 120 h.

2.5. Seed germination bioassay

Seeds of *Phalaris minor* were collected in Tunisia from wheat fields. However, seeds of *Trifolium incarnatum* and *Silybum marianum* were obtained from ECOSEM industry in Belgium. They were sterilized using 5% sodium *hypochlorite* for 2 min. Filter papers were placed in 11-cm-diameter Petri dishes and moistened with 2 mL of Tween 1% solution (which did not interfere with the different assays) for the seedling control, or with EO solutions at 0.625, 1.25, 2.5, and 5 mM for the treated seedlings. Ten seeds of *T. incarnatum*, *S. marianum* or *P. minor* were then placed immediately in Petri dishes, and three replicates were prepared for each EO concentration. All Petri dishes were randomly placed in a growth chamber at a temperature of $23 \pm 1^\circ\text{C}$, in the dark. The number of germinated seedlings was counted, and their hypocotyls and root lengths were measured after 7 days (Amri *et al.*, 2012; Ben Ghnaya *et al.*, 2013). In order to know if *R. officinalis* EO had only slowed down germination or completely inhibited it, a supplementary test was

carried out. It consisted in transferring the treated seeds from filter paper moistened with EO at 5 mM to agar solution, to check if germination might continue/resume or not. But no seed had germinated after 5 days.

2.6. Post-emergence activity of the essential oil

Another experiment was performed to study the effect of EO on 2-3-week-old *T. incarnatum*, *S. marianum*, and *P. minor* plantlets under controlled conditions (natural photoperiod supplemented with artificial light if needed, with $20 \pm 3^\circ\text{C}$ according to the sunlight. The relative humidity was $60 \pm 3\%$). Only *P. minor* seeds were sown in boxes, whereas *T. incarnatum* and *S. marianum* seeds were sown in pots. The weed seeds were sown in 11-cm-diameter pots, and the plants were watered every day. Once the weeds reached the 2-3-leaf stage, several solutions were sprayed. They consisted of 10 mL of *R. officinalis* EO at 7.5, 20, and 34 mM, formulated *R. officinalis* EO at 34 mM, adjuvants alone (as negative controls), distilled water, and a commercial biological herbicide containing 34 mM of pelargonic acid (as a positive control). Three replications were conducted for each treatment, in a completely randomized manner. Seven days after spraying, the treated weed plants were examined to assess wilting, necrosis, and chlorosis. The percentage of efficacy was calculated following the equation :

$$\text{Percentage of efficacy (\%)} = N/T * 100$$

where *N* refers to the number of necrotic or withered leaves, and *T* represents the total number of leaves.

2.7. Statistical analysis

Pre-emergence and post emergence tests were conducted using a randomized block design with 3 replications. Statistical analyses were performed with Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA). Results were examined statistically using one-way analysis of variance (ANOVA) followed by Tukey's multiple range tests. The differences between individual means were considered significant if $p < 0.05$.

3. Results

3.1 Chemical composition of *R. officinalis* essential oil

The EO obtained by hydrodistillation of dried *R. officinalis* flowers and leaves had a clear green color and emitted a pungent smell. The extraction yield was *ca.* 1.2% (w/v). The EO components identified by gas chromatography/mass spectrometry (GC/MS) are listed in Table 11. This process identified 98.71% of the compounds present in the EO. The *R. officinalis* EO was characterized by the predominance of the monoterpene class, among which 1,8 cineole, camphor, and α -pinene were the most present. This class was followed by ketones and alcohols, while esters and sesquiterpenes were found in minor quantities.

Tableau 11: Chemical constituents of the essential oil extracted from *Rosmarinus officinalis* dried leaves and flowers.

Compounds	RI ^a	RI ^b	(%) ^c
<i>Monoterpene hydrocarbons</i>			17.09
α -Thujene	928	910–935	0.31
α -Pinene	931	921–944	7.09
Camphene	950	936–965	3.09
β -Pinene	980	962–987	3.81
Myrcene	993	975–991	0.44
Phellandrene	1005	990–1009	0.10
γ -3 carene	1011	997–1027	0.27
<i>p</i> -Cymene	1026	1004–1029	1.39
γ -Terpinene	1062	1049–1069	0.41
α -Terpinene	1012	1154–1195	0.18
<i>Oxygenated monoterpenes</i>			80.19
Camphor	1143	1481–1537	12.27
1,8 cineole	1033	1021–1044	54.60
Borneol	1165	1653–1728	9.66
Terpinen 4 ol	1178	1165–1189	0.90
Terpineol	1189	1178–1203	2.76
<i>Esters</i>			0.72
Bornyl-acetate	1286	1264–1297	0.72
<i>Sesquiterpenes</i>			0.71
β -Caryophyllene	1421	1384–1430	0.62
α -Humulene	1455	1430–1466	0.04
γ Cadinene	1525	1498–1531	0.05

^a Calculated retention indexes relatively to C₈-C₂₄ n-alkanes injected in the HP 5MS column.

^b Retention indexes relatively to C₈-C₂₄ n- alkanes injected in the HP 5MS column, based on Babushok *et al.* (2011).

^c Relative quantifications were calculated by dividing the peak area of each compound by the total area of each chromatogram.

3.2 Antifungal activity of *R. officinalis* essential oil

In a dose-response bioassay, our results showed that this EO had an interesting potential at different concentrations (1, 3, and 6 mM). A rise in EO concentration increased spore germination inhibition of plant pathogens after 5 days of incubation. At the lowest EO dose, *P. italicum* was less sensitive than *F. culmorum* and *F. oxysporum*. In fact, that concentration was the least effective one. Furthermore, at 6 mM, the inhibition percentages of spore germination were very high, *i.e.* 85.99%, 100%, and 95.40% for *F. culmorum*, *F. oxysporum*, and *P. italicum*, respectively (Fig. 23).

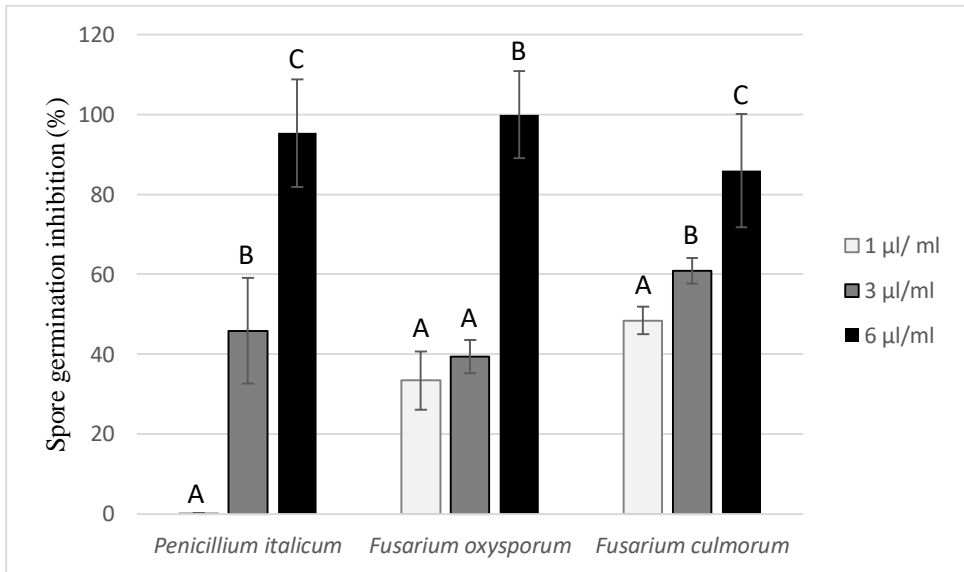


Figure 23 : Fungicidal activity of *Rosmarinus officinalis* essential oil against three plant pathogens (*Fusarium oxysporum*, *Fusarium culmorum*, and *Penicillium italicum*) after 120 h. Different letters mean significantly different results with the same strain ($p < 0.05$, Tukey's statistical test).

3.3 Herbicidal activity of *R. officinalis* essential oil under laboratory conditions

The application of EO at 5 mM completely inhibited seed germination of three weeds (*T. incarnatum*, *S. marianum* and *P. minor*) (Table 12). Moreover, EO at 1.25 and 2.5 mM caused significant delays in shoot and root growth of the same weeds after 7 days as compared to the control. As far as

germination is concerned, *T. incarnatum* proved more resistant than *S. marianum* and *P. minor* and exhibited no response at the lowest EO concentration. By contrast, the EO had strong effects on the seedling growth of these weeds, even at low concentrations.

Tableau 12: Inhibitory effects of *Rosmarinus officinalis* essential oil extracted from leaves and flowers at the vegetative stage on the germination and seedling growth of *Trifolium incarnatum*, *Silybum marianum*, and *Phalaris minor* after 7 days.

Weeds	Dose (mM)	Germination (%)	Root length (cm)	Shoot length (cm)
<i>T. incarnatum</i>	Control	100.0±0.00 ^A	4.33±0.11 ^A	3.95±0.16 ^A
	0.625	100.0±0.00 ^A	0.26±0.04 ^B	0.85±0.01 ^B
	1.25	100.0±0.00 ^A	0.26±0.02 ^B	0.63±0.01 ^B
	2.5	100.0±0.00 ^A	0.15±0.01 ^{BC}	0.31±0.02 ^C
<i>S. marianum</i>	5	0.0±0.0 ^B	0.0±0.0 ^C	0.0±0.0 ^D
	Control	86.67±8.89 ^A	1.75±0.05 ^A	2.69±0.09 ^A
<i>P. minor</i>	0.625	80.00±13.33 ^A	1.73±0.05 ^A	2.78±0.09 ^A
	1.25	70.00±6.66 ^{AB}	1.10±0.04 ^B	1.03±0.08 ^B
	2.5	43.33±4.44 ^B	0.70±0.007 ^C	0.75±0.01 ^C
	5	0.0±0.0 ^C	0.0±0.0 ^D	0.0±0.0 ^D
	Control	86.66±4.44 ^A	2.5±0.17 ^A	5.11±0.11 ^A
	0.625	56.66±4.44 ^B	2.24±0.04 ^A	4.72±0.10 ^A
	1.25	36.66±4.44 ^C	1.79±0.03 ^B	3.82±0.12 ^B
2.5	16.66±4.44 ^D	0.9±0.07 ^C	2.22±0.19 ^C	
5	0.0±0.0 ^E	0.0±0.0 ^D	0.0±0.0 ^D	

Means followed by different capital letters in each column are significantly different ($p < 0.05$, Tukey's statistical test).

3.4 Herbicidal activity of *R. officinalis* essential oil under greenhouse conditions

Studies in which EOs are applied in post-emergence conditions are scarce. For this reason, *R. officinalis* EO was sprayed on 2-3-week-old weed plants in another set of experiments to determine its post-emergence herbicidal activity. The treatment using 7.5 mM EO showed weed resistance and no visual damage. At 20 mM, the EO caused a few symptoms of injuries on *T. incarnatum* and *P. minor* (Table 13). However, at 34 mM, the EO caused more visible injuries ranging from wilting after 1 day and chlorosis after 3 days on *T. incarnatum*. Its herbicidal activity reached up to 45%. *S. marianum* was

consistently more resistant than *T. incarnatum* and *P. minor* at all concentrations. Pelargonic acid (used as positive control at 3.4%) completely punctured *T. incarnatum* and stopped *P. minor* and *S. marianum* growth. We also used the same EO in a formulated version to enhance the distribution, the coverage, and the penetration of the active molecules. It presented a high herbicidal activity, higher than the non-formulated EO, which reached 71.33% against *T. incarnatum*. Six hours after spraying the formulated EO, *T. incarnatum* and *P. minor* leaves were already wilting.

Tableau 13 : Herbicidal activity of *Rosmarinus officinalis* essential oil (EO) extracted from leaves and flowers at the vegetative stage on weeds under greenhouse conditions.

Treatment	Dose (%)	<i>Trifolium incarnatum</i>	<i>Silybum marianum</i>	<i>Phalaris minor</i>
Negative control	-	0.0±0.0 ^E	0.0±0.0 ^C	0.0±0.0 ^D
EO-free formulation	-	0.0±0.0 ^E	0.0±0.0 ^C	0.0±0.0 ^D
EO	0.75	0.0±0.0 ^E	0.0±0.0 ^C	0.0±0.0 ^D
	2	24±2.66 ^D	0.0±0.0 ^C	27.33±4.44 _C
	3.4	45±2.0 ^C	0.0±0.0 ^C	34.33±2.88 _C
Formulated EO	3.4	71.33±2.44 ^B	18±4.66 ^B	46.33±2.22 _B
Formulated pelargonic acid	3.4	100±0.0 ^A	100±0.0 ^A	100±0.0 ^A

Means followed by different letters in each column are significantly different ($p < 0.05$, Tukey's statistical test).

4. Discussion

Our results show that *R. officinalis* EO is an interesting antifungal and herbicidal agent from which a more environment-friendly alternative to

chemical herbicides might be derived. The antifungal and herbicidal activities of EOs have been widely reported in recent years (Pintore *et al.*, 2002; Salamci *et al.*, 2007; Tian *et al.*, 2012; Amri *et al.*, 2012; Kaur *et al.*, 2012; Ben Ghnaya *et al.*, 2013; Ahluwalia *et al.*, 2014; Bouabidi *et al.*, 2015; Hmiri *et al.*, 2015; Alipour & Saharkhiz, 2016; Synowiec *et al.* 2016), but to our knowledge, only a few studies have focused on their effect on post emergence when sprayed on weeds (Hazrati *et al.*, 2017). *R. officinalis* is largely used in traditional medicine (Pintore *et al.*, 2002; Ben Jemia *et al.*, 2015) and widely known for its antimicrobial and antioxidant activities (Bozin *et al.*, 2007; Celiktas *et al.*, 2007; Zaouali *et al.*, 2010), but the present study unveils its herbicidal effect in pre-emergence and post-emergence for the first time. On the other hand, GC-MS analysis of our *R. officinalis* EO extracted from dried leaves and flowers identified 19 compounds dominated by oxygenated monoterpenes including 1,8 cineole, camphor, and borneol. These results are in agreement with Zaouali *et al.* (2010), who showed that these three major components are also predominant in the Tunisian *R. officinalis* EO. However, their percentages varied between 26.0-51.2%, 4.9-29.7% and 3.3-10%, respectively. These differences in chemical composition could be related to environmental factors (the climate, the season, the soil), the genetic diversity of the species, and the geographic conditions (Ben Ghnaya *et al.*, 2013). Interestingly, the monoterpenes identified as main constituents in our EO have been described as powerful inhibitors of the seed germination and growth of several plant species (De Martino *et al.*, 2010; Barton *et al.*, 2014). These compounds also showed antifungal activity (Ben Ghnaya *et al.*, 2013; Ahluwalia *et al.*, 2014; Marei & Abdegaleil, 2018).

In addition, EOs from plants of the *Lamiaceae* family, and among them *R. officinalis*, are known for their antimicrobial activity (Hendel *et al.*, 2016). In our study, *R. officinalis* significantly inhibited the spore germination of *P. italicum*, *F. oxysporum*, and *F. culmorum*. *F. oxysporum* and *F. culmorum* have been widely documented as the most important plant pests; they cause substantial economic losses worldwide (Hollingsworth & Motteberg, 2008). *R. officinalis* EO from Greece caused a dose-dependent inhibition of the mycelial growth of five fungi (*Sclerotinia sclerotiorum*, *Phytophthora nicotianae*, *Sclerotium cepivorum*, *F. oxysporum*, and *Fusarium proliferatum*) (Pitarokili *et al.*, 2002). In addition, Sardinian *R. officinalis* EO (450 and 900 $\mu\text{L}/\text{mL}$) showed a weak activity against all tested fungi (*Botrytis cinerea*, *F. oxysporum lycopersici*, *Fusarium graminearum*, *F. culmorum*, and

Rhizoctonia solani). On the other hand, these EOs present multiple mechanisms of action due to a large number of active compounds that reduces the development of fungal resistance. For instance, a recent study confirmed that 1,8 cineole alone had a low antifungal power but showed an important synergistic effect with α -pinene (Hmiri *et al.*, 2015). These two compounds were identified in our EO. In the same line, other reports suggested that 1,8 cineole combined with terpinen-4-ol, also the major component of *Melaleuca alternifolia* EO, had a significant synergistic effect on the hyphal morphology of *B. cinerea* and its ultrastructure as compared to the treatment using either component alone. In fact, 1,8 cineole can penetrate the cell and damage cellular organelles without affecting membrane permeability. On the other hand, terpinen-4-ol destroys membrane integrity and increases permeability, resulting in ion leakage and membrane dysfunctioning. Several studies reported that EOs could cause structural and functional damage by disrupting the membrane permeability and the osmotic balance of the cell (Yu, Wang, Shao, Xu, & Wang, 2015). Other studies have shown that they can acidify the external medium and decrease ATPase and dehydrogenase activities in *Aspergillus flavus* cells (Tian *et al.*, 2012). Furthermore, EO from seeds of *Anethum graveolens* showed fungicidal activity against *Sclerotinia sclerotiorum* by inhibiting mycelial growth and sclerotial germination. This effect is the consequence of the inhibition of ergosterol synthesis, malate dehydrogenase, and succinate dehydrogenase (B. Ma *et al.*, 2015).

In parallel, to our knowledge no study had yet tackled the herbicidal activity of *R. officinalis* EO. In fact, our experiments highlighted the outstanding inhibition of three different weeds after treatment with our EO. This was seen on the percentage of germination, root growth, and hypocotyl length. In fact, 100% inhibition of germination and seedling growth was observed with our EO at 5 mM. In this context, Poonpaiboonpipat *et al.* (2013) showed that at 1 μ L and 2 μ L/Petri dish of *Cymbopogon citratus* EO, there was no significant effect on shoot or root length, but seedling length was shorter at 4 and 8 μ L/Petri dish. The strong phytotoxic activity was due to the presence of oxygenated monoterpenes, which is quite similar to that of Tunisian *Eucalyptus erthrocorys* EO, renowned for its overwhelming phytotoxic effect (Ben Ghnaya *et al.*, 2013). In this context, among 12 EOs tested on weeds, caraway, thyme, peppermint, and sage oils were classified as the most phytotoxic ones owing to the existence of oxygenated monoterpenes in a 64.1–93.3% range (Synowiec *et al.*, 2016). In line with this, among six

monoterpenes tested by Gouda *et al.* (2016), 1,8 cineole and (S)-limonene were showed to inhibit *Echinochloa crus-galli* shoot growth. The major components of EOs are very important for their biological activity, but even the minor ones could have significant synergistic effects (Synowiec *et al.*, 2016). Many other individual compounds identified in *R. officinalis*, such as α -terpineol, citronellal, citronellol, and α -pinene, have been confirmed to have phytotoxic activity (Zhang, An, Wu, Liu, & Stanton, 2014). In contrast, among 25 EOs, only those containing volatile phenolic compounds such as thymol, carvacrol, eugenol, alcohols or ketones, showed strong phytotoxic effect on different weed seeds, even though the mode of action of all these compounds has not yet been detailed and a number of effects and hypotheses have been reported by many authors. Several authors assume that EOs act by causing biochemical and physiological changes in seedling growth (De Martino *et al.*, 2010). For instance, *Cymbopogon citrates* EOs notably slowed down α -amylase activity in *E. crus-galli* seeds (Poonpaiboonpipat *et al.*, 2013). Another clear example is *Artemisia* sp. EO: it induced reactive oxygen species production, which in turn caused damage resulting in lipid peroxidation, decreased membrane fluidity, and finally increased membrane leakiness and inactivated receptors, enzymes and ion channels (Kaur *et al.*, 2012b). Moreover, 1,8 cineole inhibited root growth and stopped DNA synthesis through several steps (Koitabashi *et al.*, 1997).

We applied *R. officinalis* EO not only in pre-emergence tests but also for the first time in post-emergence tests, by spraying it on weeds under greenhouse conditions. Based on the visual damage induced by this EO on weeds three days after spraying, herbicidal properties were noticed. Necrosis and wilting leaves were observed at a concentration of *R. officinalis* EO starting from 20 mM. Similar results showed that the spraying of *Cymbopogon citratus* EO from 1.25 mM to 10 mM on *E. crus-galli* leaves caused wilting, and the leaves exhibited desiccation symptoms. In addition, this EO decreased the chlorophyll a, b and carotenoid contents, and caused electrolyte leakage, indicating membrane disruption and loss of integrity (Poonpaiboonpipat *et al.*, 2013). Monoterpenes, which are present at 80.19% in our *R. officinalis* EO, may affect plant photosynthesis, energy metabolism, and the biosynthesis of secondary metabolites such as phenolic compounds (Gouda *et al.*, 2016). In addition, it has been confirmed that the penetration of monoterpenes through the cell wall and cell membrane can cause cellular potassium leakage that inhibits glucose-dependent respiration. A recent study showed that the

spraying of a nano-emulsion of *Satureja hortensis* EO reduced the weed chlorophyll content, and increased electrolyte leakage and cell membrane disruption (Hazrati *et al.*, 2017).

We investigated a formulation of *R. officinalis* EO as a bioherbicide for the first time, based on the following observations: (1) as *R. officinalis* EO is lipophilic, it does not dissolve well in water; (2) in the same line, the reported herbicidal effect of *Satureja hortensis* EO in the absence of tween 20 was lower on control weeds; and (3) EOs contain terpenoids that are volatile, thermolabile, and may be easily oxidized and hydrolyzed (Pavela *et al.*, 2016). For these reasons, we used an emulsifier providing better stability, efficacy and persistence for the formulation. An ionic surfactant reduced the effective concentration of eucalypt oil for a high herbicidal activity against *P. minor* (Batish *et al.*, 2007). Based on that, a recent study showed that a formulation containing palm oil, tween 20 and span 80 improved the herbicidal activity of metabolites from *Phoma* sp. (Toderò *et al.*, 2018).

To our knowledge, this is the first report that links the chemical composition of Tunisian *R. officinalis* EO to its fungicidal and bio-herbicidal effects on plant pathogens and weeds, respectively. Moreover, the formulation of the bio-herbicide based on Tunisian *R. officinalis* EO was attempted in this work for the first time. Hence, this work opens new perspectives on the application of Tunisian *R. officinalis* EOs as a novel biocontrol strategy against harmful plant pathogens and weeds. It also paves the way for new strategies and pathways for the biopesticide industry to create alternative chemical pesticides designed to be less harmful to the environment and human health than current ones. For agronomic applications, we found that *R. officinalis* EO could be used as a biofungicide at low concentrations between 1 mM and 6 mM without any phytotoxic effect in post-emergence tests. At concentrations higher than 20 mM, this EO can be used as a post-emergence bioherbicide. According to our preliminary results, the use of EOs in the formulation of bioherbicides can offer new prospects for the sustainable production and practical use of EOs. To go further in the experiments, it could be really interesting to determine the modes of action of *R. officinalis* EO on weeds and fungi and try to improve the effectiveness and stability of the bioherbicidal *R. officinalis* EO formulation.

References

- Ahluwalia V, Sisodia R, Walia S, Sati OP, Kumar J, Kundu A, 2014. Chemical analysis of essential oils of *Eupatorium adenophorum* and their antimicrobial, antioxidant and phytotoxic properties. *J Pest Sci* 87: 341-349.
- Alipour M, Saharkhiz MJ, 2016. Phytotoxic activity and variation in essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) during different phenological growth stages. *Biocatal Agric Biotechnol* 7: 271-278.
- Amri I, Gargouri S, Hamrouni L, Hanana M, Fezzani T, Jamoussi B, 2012. Chemical composition, phytotoxic and antifungal activities of *Pinus pinea* essential oil. *J Pest Sci* 85: 199-207..
- Angioni, A.L.A., Arra, A.N.B., Ereti, E.L.C., Arile, D.A.B., Oroneo, V.A.C., Abras, P.A.C., Sanita, D., Porcell, V., 2004. Chemical Composition , Plant Genetic Differences , Antimicrobial and Antifungal Activity Investigation of the Essential Oil of *Rosmarinus officinalis* L . *J.Agric.Food.Chem*, 52, 3530-3535.
- Araniti, F., Mancuso, R., Lupini, A., Giofrè, S., Sunseri, F., Gabriele, B., Abenavoli, M., 2015. Phytotoxic Potential and Biological Activity of Three Synthetic Coumarin Derivatives as New Natural-Like Herbicides. *Molecules*, 20, 17883-17902.
- Babushok, V.I., Linstrom, P.J., Zenkevich, I.G, 2011. Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *J. Phys. Chem.*
- Barton, A.F.M., Clarke, B.R., Dell, B., Knight, A.R., 2014. Post-emergent herbicidal activity of cineole derivatives *J. Pest Sci* 531-541.
- Batish, D.R., Singh, H.P., Setia, N., Kohli, R.K., Kaur, S., Yadav, S., 2007. Alternative control of littleseed canary grass using eucalypt oil. *Agron. Sustain. Dev.* 27, 171-177.
- Ben Ghnaya, A., Hanana, M., Amri, I., Balti, H., Gargouri, S., Jamoussi, B., Hamrouni, L., 2013. Chemical composition of *Eucalyptus erythrocorys* essential oils and evaluation of their herbicidal and antifungal activities. *J. Pest Sci.* 86, 571-577.
- Ben Jemia, M., Tundis, R., Pugliese, A., Menichini, F., Senatore, F., Bruno, M., Kchouk, M.E., Loizzo, M.R., 2015. Effect of bioclimatic area on the composition and bioactivity of Tunisian *Rosmarinus officinalis* essential oils. *Nat. Prod. Res.* 29, 213-22.

- Bouabidi, W., Hanana, M., Gargouri, S., Amri, I., Fezzani, T., Ksontini, M., Jamoussi, B., Hamrouni L, 2015. Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis* L. essential oils. Nat. Prod. Res. 29, 864-8.
- Boubaker, H., Karim, H., Hamdaoui, A.E., Msanda, F., Leach, D., Bombarda, I., Vanloot, P., Abbad, A., Boudyach, E.H., Ben, A.A., 2016. Chemical characterization and antifungal activities of four *Thymus* species essential oils against postharvest fungal pathogens of citrus. Ind. Crop. Prod. 86, 95-101.
- Bozin, B., Dukic, N.M., Samojlik, I., JOvin, E., 2007. Antimicrobial and Antioxidant Properties of Rosemary and Sage (*Rosmarinus officinalis* L . and *Salvia officinalis* L ., Lamiaceae) Essential Oils. J.Agric.Food.Chem, 7879-7885.
- Celiktas, O.Y., Kocabas, E.E.H., Bedir, E., Ozek, T., Baser, K.H.C., 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis* , depending on location and seasonal variations. Food Chem. 100, 553-559.
- De Martino, L., Mancini, E., De Almeida, L.F.R., De Feo, V., 2010. The antigerminative activity of twenty-seven monoterpenes. *Molecules* 15, 6630-6637.
- Giamperi, L., Fraternali, D., Ricci, D., 2011. The In Vitro Action of Essential Oils on Different Organisms. J. Essent.Oil Res 14,312-318.
- Gouda, N.A.A., Saad, M.M.G., Abdelgaleil, S.A.M., 2016. PRE and POST Herbicidal Activity of Monoterpenes against Barnyard Grass (*Echinochloa crus-galli*). Weed Sci. 64, 191-200 .
- Hazrati, H., Saharkhiz, M.J., Niakousari, M., Moein, M., 2017. Natural herbicide activity of *Satureja hortensis* L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. Ecotoxicol. Environ. Saf. 142, 423-430.
- Hendel N, Larous L, Belbey, L., 2016. Antioxidant activity of rosemary (*Rosmarinus officinalis* L .) and its in vitro inhibitory effect on *Penicillium digitatum*. *International Food Research Journal* 23, 1725-1732.
- Hmiri, S., Harhar, H., Rahouti, M., 2015. Antifungal activity of essential oils of two plants containing 1 , 8-cineole as major component : *Myrtus communis* and *Rosmarinus officinalis*. J. Mater. Environ. Sci. 6, 2967-2974.

- Hollingsworth, C.R., Motteberg, C.D., 2008. Agronomic and Economic Responses of Spring Wheat to Management of Fusarium Head Blight. Plant disease 1339-1348.
- Kaddes, A., Parisi, O., Berhal, C., Ben kaab, S., Fauconnier, M.F., Nasraoui, B., Jijakli, M.H., Massart, S., De Clerck, C., 2016. Evaluation of the effect of two volatile organic compounds on barley pathogens *Molecules* 21, 1-10.
- Kaur, S., Singh, H.P., Batish, D.R., Kohli, R.K., 2012. *Artemisia scoparia* essential oil inhibited root growth involves reactive oxygen species (ROS)-mediated disruption of oxidative metabolism: In vivo ROS detection and alterations in antioxidant enzymes. *Biochem. Syst. Ecol.* 44, 390-399.
- Koitabashi, R., Suzuki, T., Kawazu, T., Sakai, A., 1997. 1,8-Cineole Inhibits Root Growth and DNA Synthesis in the Root Apical Meristem of *Brassica campestris*. *J.Plant.Res.* 110:1-6.
- Koul, O., Walia, S., Dhaliwal, G.S., 2008. Essential oils as green pesticides: Potential and constraints. *Biopestic. Int.* 4, 63-84.
- Ma, B., Ban, X., Huang, B., He, J., Tian, J., Zeng, H., Chen, Y., Wang, Y., 2015 Interference and mechanism of dill seed essential oil and contribution of carvone and limonene in preventing sclerotinia rot of rapeseed. *PLoS One* 10, 1-15.
- Marei GIK, Abdelgaleil SAM, 2018. Antifungal potential and biochemical effects of monoterpenes and phenylpropenes on plant. *Plant Protect Sci* 54: 9-16.
- Pavela, R., Benelli, G., 2016. Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. *Trends Plant Sci.* 21, 1000-1007.
- Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi F., Chessa, M., Cerri, R., Casanova, J., 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L . oils from Sardinia and Corsica. *Flavour and fragrance journal*, 17, 15-19.
- Pitrokili, D., Maria, C., Niki P.P, Olga, T., 2002. Composition and antifungal activity on soil-borne pathogenes of the essential oil of *salvia sclarea* from Greece. *J Agri Food Chem* 50:6688-6691.
- Poonpaiboonpipat, T., Pangnakorn, U., Suvunnamek, U., Teerarak, M., Charoenying, P., Laosinwattana, C., 2013. Phytotoxic effects of essential oil from *Cymbopogon citratus* and its physiological mechanisms on barnyardgrass (*Echinochloa crus-galli*). *Ind. Crops Prod.* 41, 403-407.

- Salamci, E., Kordali, S., Kotan, R., Cakir, A., Kaya, Y., 2007. Chemical compositions , antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var . *chiliophyllum*. *Biochem.System.Ecol* 35, 569-581.
- Schütte, G., Eckerstorfer, M., Rastelli, V., Reichenbecher, W., Restrepo-Vassalli, S., Ruohonen-Lehto, M., Saucy, A.G.W., Mertens, M.: Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. *Environ. Sci. Eur.* 29, 5.
- Synowiec, A., Kalemba, D., Drozdek, E., Bocianowski, J., 2016. Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. *J Pest Sci* 90 (1): 407-419.
- Tian, J., Ban, X., Zeng, H., He, J., Chen, Y., Wang, Y., 2012. The mechanism of antifungal action of essential oil from dill (*Anethum graveolens* L.) on *Aspergillus flavus*. *PLoS One* 7 (1): e30147,.
- Todero, I., Confortin, T.c., Luft, L., Brun, T., Ugalde, G.A., Almeida, T.C.D., Arnemann, J.A., Zobot, G.L., Mazutti, M.A., 2018 : Formulation of bioherbicide with metabolites from *Phoma* sp. *Scientia Horticulturae* 241, 285-292
- Uremis, I., Arslan, M., Sangun, M.K., 2009. Herbicidal activity of essential oils on the germination of some problem weeds. *Asian J. Chem.* 21, 3199-3210.
- Usanmaz, A., Cakir, A., Komaki, A., Ercisli, S., 2016. Antifungal and herbicidal effects of Fruit essential oils of four *Myrtus communis* Genotypes. *Chem. Biodiversity* 77-84.
- Yu, D., Wang, J., Shao, X., Xu, F., Wang, H., 2015. Antifungal modes of action of tea tree oil and its two characteristic components against *Botrytis cinerea* J. *Applied Microbiology* 1364-5072.
- Zaouali, Y., Bouzaine, T., Boussaid, M., 2010. Essential oils composition in two *Rosmarinus officinalis* L . varieties and incidence for antimicrobial and antioxidant activities. *Food.Chem.tox* 48, 3144-3152.
- Zhang, J., An, M., Wu, H., Liu, D.L., Stanton, R., 2014. Phytotoxic activity and chemical composition of aqueous volatile fractions from Eucalyptus species. *PLoS One* 9 (3): e93189.

4

Etude du mode d'action des composés bioactifs

***Cynara cardunculus* crude extract as a powerful natural herbicide and insight into the mode of action of its bioactive molecules**

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Le chapitre 4 s'intéresse à l'étude des modes d'actions des extraits végétaux et l'interaction de leurs molécules bioactives identifiées avec la membrane plasmique de la plante. En effet, l'utilisation d'herbicides chimiques pourrait non seulement induire des impacts négatifs sur l'environnement, les animaux et la santé humaine, mais également augmenter la résistance des adventices aux herbicides. Dans ce contexte, l'utilisation d'extraits de plantes pourrait constituer une alternative intéressante et naturelle aux produits chimiques. Il est donc important de comprendre le mode d'action de leurs composés bioactifs. C'est pourquoi nous avons étudié l'effet herbicide de l'extrait brut de *C. cardunculus* en termes d'inhibition de la croissance des plantules de mauvaises herbes et leur impact sur les paramètres physiologiques des plantules traitées, tels que la conductivité, le poids sec, la fluorescence et les paramètres biochimiques liés au stress oxydatif, tels que le H₂O₂ et le MDA. Nous avons également remarqué que l'extrait brut de *C. cardunculus* induit un stress oxydatif chez les plantes traitées et perturbe par conséquent les fonctions physiologiques et biochimiques des cellules végétales. Pour aller plus loin, nous avons étudié pour la première fois la capacité de ces composés bioactifs à interagir avec la membrane plasmique. Nos résultats montrent que la naringénine et la myricitrine seules peuvent pénétrer à travers la partie polaire de la membrane mais elles ne semblent pas pouvoir traverser la partie apolaire. Notre hypothèse repose sur le fait que cette interaction pourrait perturber l'organisation des lipides dans la membrane de la plante et bloquer ainsi certains processus physiologiques et biochimiques utiles à la croissance de la plante.

Abstract

The use of chemical herbicides could not only induce negative impacts on the environment, animals and human health but also increase the weeds resistance to herbicides. In this context, the use of plant extracts could be an interesting and natural alternative to chemical products. It is thus of importance to know more about them and to understand the mode of action of their bioactive compounds. This is why, we have studied the herbicidal effect of *Cynara cardunculus* crude extract in term of inhibition of weeds 'seedling growth and their impact on physiological parameters of treated plantlets, like conductivity, dry weight, fluorescence and biochemical parameters linked to oxidative stress such as H₂O₂ and MDA. We have observed that *C. cardunculus* crude extract induce oxidative stress in the treated plants and consequently disturb the physiological and biochemical functions of the plant cells. To go further we have investigated for the first time the ability of the bioactive compound of our crude extract to interact with plasma membrane. Our results show that naringenin and myricitrin only can penetrate through the polar part of the membrane. They do not seem to be able to cross the apolar part. Our hypothesis is that this interaction could disturb the lipid organisation in the plant membrane and consequently block some physiological and biochemical processes useful for plant growth.

1. Introduction

In the agricultural field, weeds are the first cause of yield reduction (about 32%), far ahead of pathogens (15%) [1]. They are constantly competing with crops for water, light and nutrient resources and therefore lead to huge economic losses [2]. Among the weed control methods, chemical herbicides are the most used ones. However, it is confirmed that their intensive use could induce negative impacts on the environment, animals and human health and increase the weed resistance to herbicides. As an example, 210 species of weeds became resistant to herbicides in 2015 [3,4]. Moreover, it has been nearly 20 years that no herbicide with a new target site came on the market. It is hence of primary importance to discover natural compounds with new herbicide target sites [5]. In this context, phenolic crude extracts containing several compounds, could be a solution to combat weed resistances, as they usually have multisite action, which is not the case for synthetic herbicides [3].

Plant extracts, which were traditionally used for medical, nutritional and even artisanal purposes [6] might be an alternative to develop natural herbicides for a sustainable agriculture [7]. They have been studied for many years for their fungicidal and bactericidal properties, but few studies have focused on their herbicidal properties [8]. In this context, a recent study showed that among 10 plant extracts, *C. cardunculus* crude methanolic extract had the best herbicidal activity in post-emergence [9]. *C. cardunculus* leaves are known to be a good source of phenolic compounds and to have therapeutic potential as antidiabetic and antimicrobial agent [6].

The phytotoxic effect of this phenolic extract is probably due to the presence of an aromatic ring comprising several hydroxyl groups in its structure [10] but its mechanism of action is not clear yet [11]. One possibility is that it could disturb the plasma membrane and therefore cause the initial and basic effects related to oxidative stress. The latter could affect several cell functions, and finally leads to the destruction and death of cells [12-16]. The cuticle is considered as a first barrier for a molecule to penetrate into the plant but little attention was given to the initial contact between these phenolic compounds and the plasma membrane. The latter is a complex dynamic entity.

The herbicidal activity of plant extracts has been already documented [17; 18] but mostly consisted in determining the presence or not of inhibitory effect and in characterizing the bioactive compounds. However, to our best knowledge, no study has focused on the effect of crude extracts and their individual bioactive molecules on the weeds. In this context, our study aims to characterize the physiological responses (such as conductivity, dry weight, fluorescence and biochemical parameters linked to oxidative stress) of *Trifolium incarnatum* plants treated with *C. cardunculus* crude extract and their individual phenolic compounds compared to conventional herbicides in order to investigate their mode of action.

2. Material and methods

2.1 Plant material and preparation of formulated *C. cardunculus* crude extract

As described by [9], aerial parts of *C. cardunculus* were collected in its vegetative stage in March 2016 from Enfidha, located in the North of Tunisia. Phenolic extract of *C. cardunculus* was obtained by stirring 10 g of plant dry powder with 100 ml of methanol (Emplura EMD Millipore Corporation filiale de Merck KGaA) for 30 min. Methanol was then eliminated using a rotavapor in a vacuum at 45°C, and residues were redissolved in a solution of tween 1%. Extract was kept for 24 h at 4 °C, filtered through a Whatman N° 4 filter paper and stored at 4 °C. Afterwards, the extract was formulated following the method developed in [7] in order to ease the penetration of active molecules through epicuticular waxes.

2.2 Evaluation of the herbicidal effect of *C. cardunculus* crude extract and its bioactive compounds

2.2.1 Evaluation of post-emergence activity of whole crude extract under greenhouse conditions

Seeds of *T. incarnatum* were obtained from ECOSEM company in Belgium. The effect of formulated *C. cardunculus* extract was studied on 2-3-week-old plantlets grown under controlled conditions (natural photoperiod supplemented with artificial light if needed, temperature of $20 \pm 3^\circ\text{C}$ according to the sunlight, and relative humidity of $60 \pm 3\%$). The weeds'

seeds were sown in 11cm-diameter pots so that the whole surface of the ground was covered, and the plants were watered every day. Once the weeds reached the 2-3-leaf stage, several solutions were sprayed. They consisted of 10 ml of non-formulated *C. cardunculus* crude extract at 31 g/L, formulated *C. cardunculus* crude extract at 31 g/L, adjuvants used in the formulation alone (as negative control) and distilled water. In addition, commercial chemical and biological herbicides containing glyphosate at 7.5 g/L and pelargonic acid at 31 g/L respectively were used as positive controls. Three replications were conducted for each treatment, in a completely randomized manner. The treated weed leaves were examined to assess several physiological parameters: dry weight, induced chlorophyll fluorescence, MDA, hydrogen peroxide production and electrical conductivity.

- **Dry weight**

The plants were collected 6 hours or 3 days after the spraying, weighted and placed in an oven at 80°C for 3 days then weighted again. The percentage of dry weight was calculated following equation 1:

$$\text{Percentage of dry weight (\%)} = \frac{\text{weight of leaves before incubation (6H or 3 days)}}{\text{weight of leaves after incubation}}, \quad (1)$$

- **Induced Chlorophyll Fluorescence**

Here again, treated leaves were collected 6 hours or 3 days after spraying. The effect of the different herbicidal compounds on photosynthesis was tested using a HandyPEA fluorimeter (Hansatech Instruments, Pentney, Norfolk, UK). The instrument was set on Kinetic Mode and adjusted so that the initial Ft (instantaneous fluorescence signal) value in the control samples was approximately 210. The other parameters were optimized as described in [16].

- **Measurement of MDA**

The melondialdehyde (MDA) is produced during lipid peroxidation and can be used as a marker of oxidative stress. Its concentration in the treated leaves was measured three days after the treatment using the protocol of [17] with some modifications. In brief, 100 mg of leaves treated with the herbicidal compounds were crushed in TCA (10 ml, 0.1%, w/v) and centrifuged at 10,000 x g for 10 min. One milliliter of the supernatant was added to 4 ml of

thiobarbaturic acid (0.5%, w/v, in 20%, w/v, TCA). The mixture was heated at 95 °C for 30 min, cooled over ice, and then centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was recorded at 532 nm and corrected for non-specific absorbance at 600 nm. The presence of MDA in the treated leaves is detected by a pink coloring. Its content was calculated using $\epsilon = 155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed in nmol. g^{-1} .

- **Measurement of electrical conductivity**

Leaves of *T. incarnatum* collected for analysis three days after spraying were also examined for their electrical conductivity, as it is an indicator of cellular damage. The conductivity of each treated leaves was measured with a conductivity meter Fluorescence Induction Monitor (FIM 1500, Analytical Development Company Limited, ADC).

- **Measurement of Hydrogen peroxide (H_2O_2)**

H_2O_2 is produced during oxidative stress. Its content in the treated leaves was determined using the protocol described in [18]. Treated leaves were collected after 3 days, extracted with trichloroacetic acid (TCA, 5 ml, 0.1%, w/v) in an ice bath, and the homogenate was centrifuged at 12,000 x g for 15 min. 0.5 ml of supernatant was then mixed with 0.5 ml phosphate buffer (pH 7) and 1 ml of potassium iodide (1 M). The absorbance of this reaction mixture was measured at 390 nm. H_2O_2 content was determined using $\epsilon = 0.28 \text{ }\mu\text{M}^{-1} \text{ cm}^{-1}$ and expressed in nmol g^{-1} .

2.2.2 Evaluation of post-emergence activity of crude extract's major compounds under greenhouse conditions

Another experiment was performed to study the post-emergence activity of the major compounds contained in *C. cardunculus* crude extract: myricitin, quercetin and naringenin [9]. These compounds were formulated in the same way than the whole crude extract, alone or in combination. The concentrations of the compounds were chosen to be in accordance with their concentration in the crude extract (myricitrin at 60 $\mu\text{g/ml}$, querecetin at 250 $\mu\text{g/ml}$ and naringenin at 100 $\mu\text{g/ml}$). Formulated myricitrin, quercetin and naringenin alone and myrcitrin combination with querecetin at 250 $\mu\text{g/ml}$ and naringenin at 100 $\mu\text{g/ml}$ (Sigma, Belgium) were sprayed on *T. incarnatum* plantlets at 2-3-leaf stage. The formulation compounds were also tested alone. Three repetitions were made in each case. Three days after

spraying, the treated leaves of *T. incarnatum* were examined to assess wilting, necrosis, and chlorosis. The percentage of efficacy was calculated for each mix following equation 2:

$$\text{Percentage of efficacy (\%)} = N/T * 100, \quad (2)$$

where N refers to the number of necrotic or withered leaves, and T represents the total number of leaves.

2.2.3 Evaluation of pre-emergence activity under laboratory conditions

T. incarnatum seeds were sterilized using 0.5 % sodium hypochlorite for 2 min. *C. cardunculus* crude extract was solubilized in tween 1% and then diluted with distilled water to the desired concentrations (0.75, 1.7, 3.4, 6.8 and 10 g/L). Filter papers were placed in 11cm diameter Petri dishes and moistened with 2 ml of Tween 1 % solution only (which did not interfere with the different assays) for the negative control, and with *C. cardunculus* crude extract for the treated seedlings.

The major synthetic phenolic compounds of the crude extract (naringenin, myricitrin quercetin) (sigma, Aldrich) were tested individually under the same laboratory conditions in a separate experiment. They were solubilized in methanol 1% and then diluted with distilled water. The concentrations of these molecules were the same than in the post emergence experiment. In this case, filter paper was moistened with 2 ml of methanol 1 % solution (which did not interfere with the experiment) as a negative control, and with the phenolic compound solutions for the treated seedlings.

For each treatment, ten seeds of *T. incarnatum* were placed in Petri dishes and three replicates (3 Petri dishes) were made for each solution. All Petri dishes for these experiments were randomly placed in a growth chamber at $23 \pm 1^\circ\text{C}$, in darkness. The hypocotyls and root lengths were measured after 7 days and the inhibition rate of the roots and hypocotyls length was calculated.

2.3 In silico interaction of phenolic compounds with biomimetic plant plasma membranes

The 3D structures of the phenolic compounds were constructed using HyperChem software (Hypercube, Inc.) (Figure 24). The molecular geometry was optimized with the steepest-descent method using the MM+ force field,

and a systematic analysis of the torsion angles using the structure tree method was performed as described previously in [18]. The most probable structure corresponding to the lowest conformational energy was used for further calculations.

The insertion of the molecule into an implicit bilayer was computed by the IMPALA procedure as described in [19]. Briefly, an implicit membrane is described as a continuous medium whose properties vary along the axis perpendicular to the bilayer plane (z axis). The membrane properties are represented by energy restraints. The phenolic molecule is systematically moved along the z axis by 1 \AA steps, from one side of the membrane to the other and the restraints are calculated for each position.

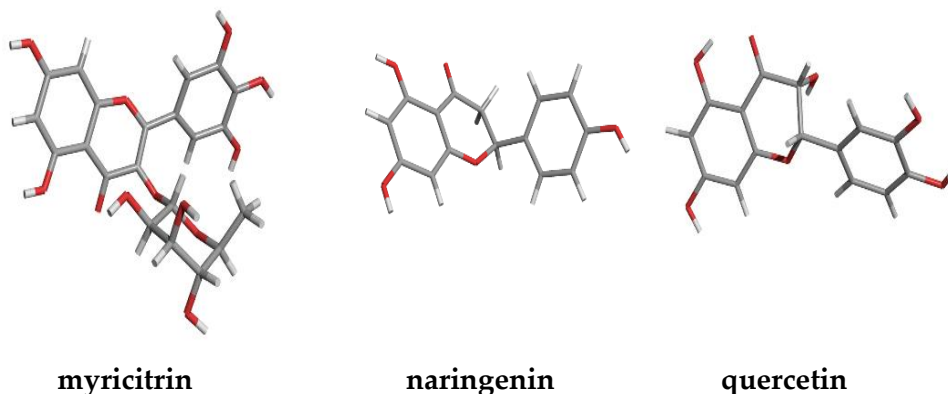


Figure 24: Chemical 3D structure of the three phenolic compounds studied

2.4 Statistical analysis

Statistical analysis was performed with Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA). Results were examined statistically using one-way analysis of variance (ANOVA) followed by Tukey multiple range tests. The differences between individual means were considered significant if $p < 0.05$.

3. Results

3.1 Evaluation of post-emergence activity under greenhouse conditions

The post-emergence tests showed that the spraying of formulated *C. cardunculus* crude extract increased the leaves dry weight percentage of *T. incarnatum* compared with both negative control (formulation compounds and distilled water) and *C. cardunculus* without formulation (Figure 25).

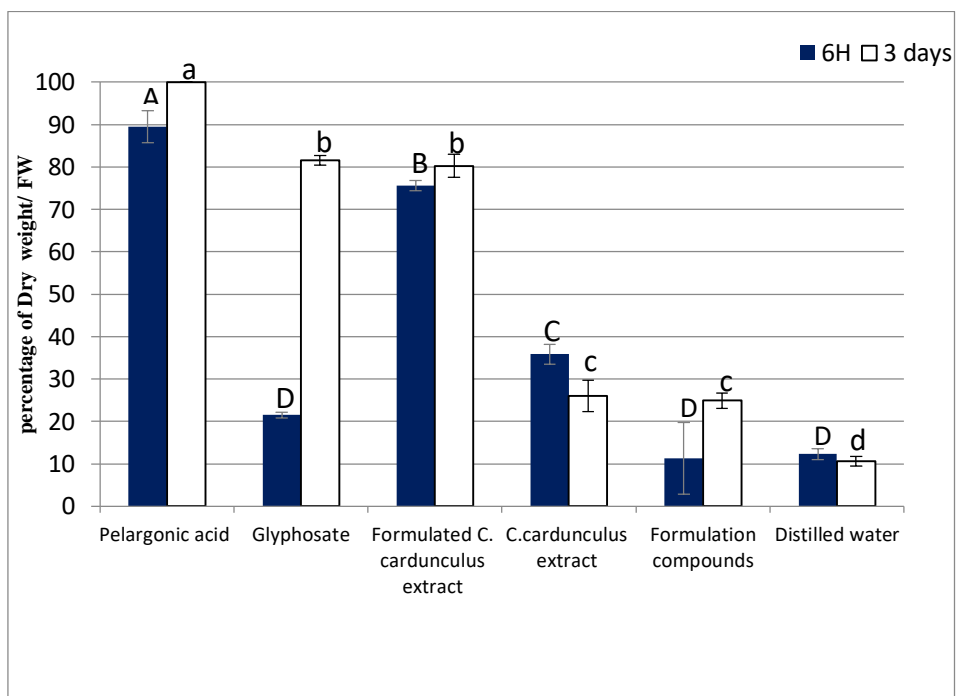


Figure 25 : Percentage of dry weight of *T. incarnatum* leaves 6 hours and 3 days after the treatment. *C. cardunculus* crude methanolic extract with and without formulation at 31 g/L, biological and chemical herbicide in the commercial concentration at the market (31g/L and 7g/L respectively) herbicide based on pelargonic acid at 34 g/L (the same concentration in the market), formulation contained only vegetable oil and adjuvants. Values in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

The dry weight percentages of leaves treated by the formulated extract indeed reached 80%, while the dry weight percentages were 26% for the leaves treated with the non-formulated *C. cardunculus* extract and 24% for the formulation without extract. On the other hand, we observed that pelargonic acid affected the dry weight more than formulated *C. cardunculus*. For glyphosate, the effect was significant after 3 days but nothing can be detected within 6 hours after spraying. The fluorescence of *T. incarnatum* leaves treated by formulated *C. cardunculus* crude extract was also measured (Figure 26).

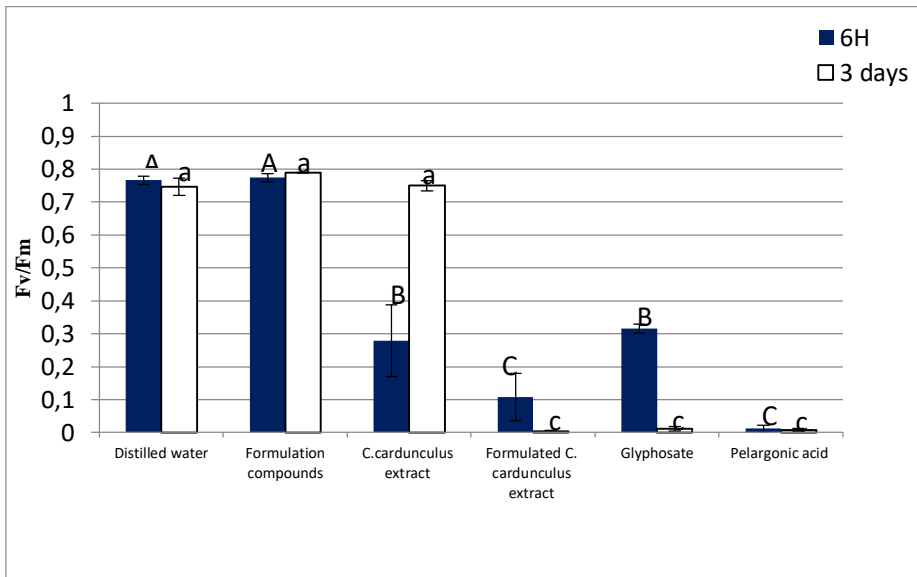


Figure 26 : Fluorescence of leaves *Trifolium incarnatum* after 6 hours and 3 days of treatment *C. cardunculus* crude methanolic extract with and without formulation at 31 g/L, fraction 2 with and without formulation at 20 g/L isolated from methanolic crude extract of *C. cardunculus* plant extract, biological and chemical herbicide in the commercial concentration at the market (31g/L and 7,5g/L respectively) herbicide based on pelargonic acid at 34 g/L (the same concentration in the market), formulation contained only vegetable oil and adjuvant. Values in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

Fluorescence analysis is often used in several studies to investigate the physiological aspects of photosynthesis and characterize plant photosynthetic performance [16].

The crude extract decreased by 85% the fluorescence of the leaves even after 6 hours in comparison with untreated leaves. The statistical analysis showed that this effect is greater than that in to the glyphosate treatment (diminution by 58% with regard to non-treated leaves). In addition, we observed that the extract without formulation does not affect the fluorescence after 6h but well after 3 days.

In order to determine the oxidative response towards the herbicidal activity of formulated *C. cardunculus* extract, several parameters were assessed. MDA, conductivity and H₂O₂ levels of *T. incarnatum* leaves sprayed with this extract were compared to the controls (Table 14).

Tableau 14 : Physiological parameters of leaves treated with herbicidal compounds. The formulation contained vegetable oil and adjuvants. *C. cardunculus* extract was used at 31 g/L and glyphosate was used at its commercial concentration (7,5 g/L).

Parameters	Distilled water	Formulation compounds	Formulated <i>C. cardunculus</i> extract	Glyphosate
MDA (nmol/gFM)	4,95 ± 3,05 B	5,59 ± 5,21 B	42,80 ± 8,71 A	56,77 ± 9,76 A
H ₂ O ₂ (nmol/gFM)	1,18 ± 0,79 B	2,25 ± 1,37 B	17,63 ± 2,13 A	4,91 ± 0,92 B
C(μs/cm)	188,20 ± 8,8 B	300,00 ± 13,33 B	961,50 ± 125,66 A	915,00 ± 33,33 A

The results showed that *C. cardunculus* extract significantly increases H₂O₂, conductivity levels and MDA content in leaves, reaching 17.6 nmol/gMF, 961,5 μs/cm and 42,8 nmol/gMF respectively. Statistical analysis indicated that H₂O₂ level in leaves treated with the formulated extract is higher than that in leaves treated with the commercial chemical herbicide. For conductivity and MDA, the effect was similar compared to the positive treatment. On the other hand, the co-formulants did not show any effect on this physiological parameter, assessing that the addition of these co-formulants does not affect the activity of *C. cardunculus* crude extract.

The effects of the main individual compounds of our crude extract on *T. incarnatum* were also studied (Figure 27).

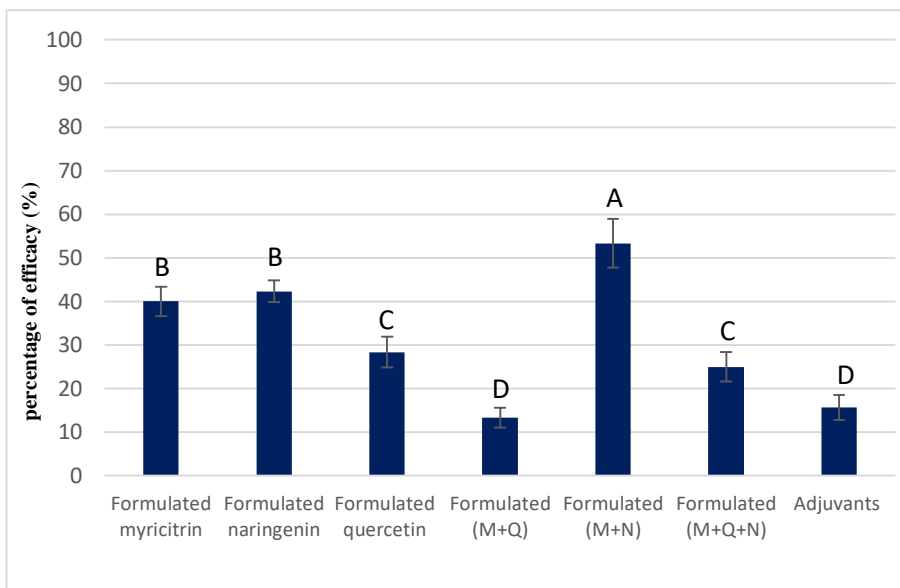


Figure 27 : Post-emergence activity of formulated myricitrin at 60 $\mu\text{g/ml}$ and its combination with quercetin at 250 $\mu\text{g/ml}$ and naringenin at 100 $\mu\text{g/ml}$ on *T. incarnatum* after 5 days. The formulation contained vegetable oil and adjuvants. Values in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

Myricitrin and naringenin showed phytotoxic effects on leaves of *T. incarnatum* with a necrosis percentage of 40 % and 42% respectively for treated leaves. In the case of quercetin, a lower herbicidal (28% of necrosis) was observed compared to the two other molecules. When combined, quercetin decreased the herbicidal effect of myricitrin by 13% while the combination with naringenin increased it by 11%.

3.2 Evaluation of pre-emergence activity under laboratory conditions

The pre-emergence tests showed that the crude extract of *C. cardunculus* strongly inhibits the seedling growth of *T. incarnatum* (Figures 28 and 29).

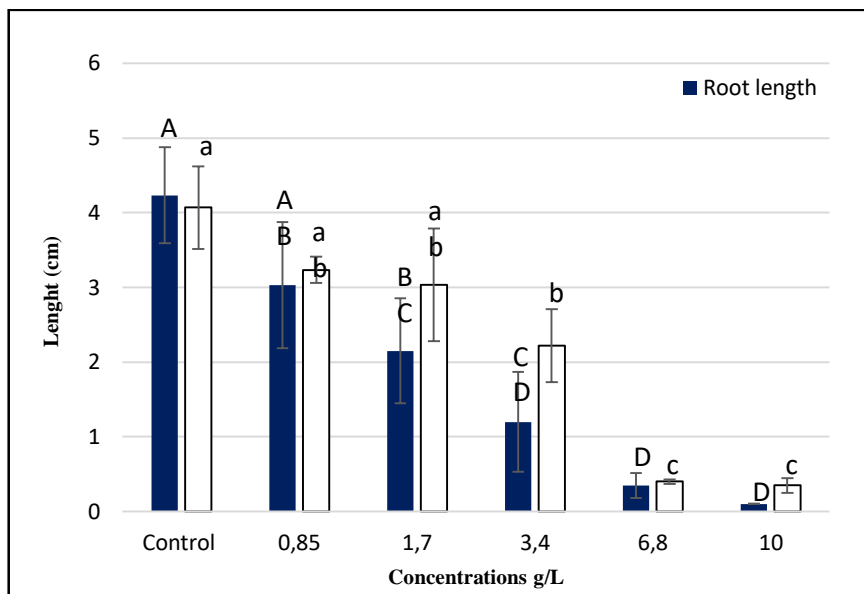


Figure 28 : Phytotoxic effect of *C. cardunculus* crude extract in pre-emergence at different concentrations (0.85, 1.7, 3.4, 6.8 and 10 g/L) on *T. incarnatum* seedling growth. Values in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

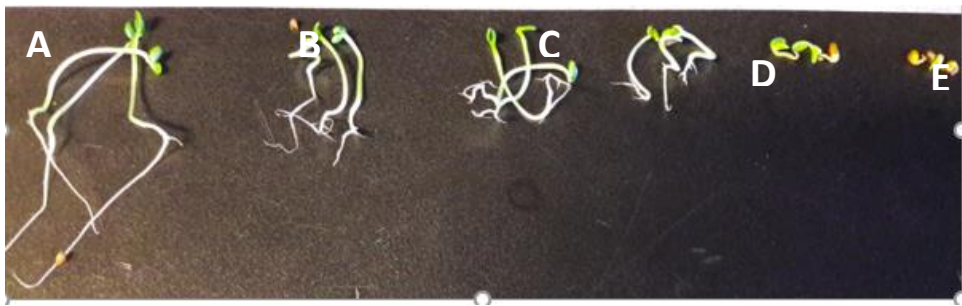


Figure 29 : Inhibitory effects of *C. cardunculus* plant extract in pre emergence, on seedling growth of *T. incarnatum* at different concentrations (A: tween 1%, B 0.85 g/L, C: 1.7 g/L, D: 3.4 g/L, E: 6.8 g/L, F: 10 g/L).

This inhibition reached up to 97% and 91 % respectively for roots and hypocotyl growth after the application of *C. cardunculus* crude extract at 10 g/L. In addition, the apparition of secondary roots was observed in the treated plants. Concerning the main compounds, only myricitrin decreased

significantly the seedling growth of *T. incarnatum*, with a reduction of 62% for the roots and 26% for the hypocotyls (Figure 30).

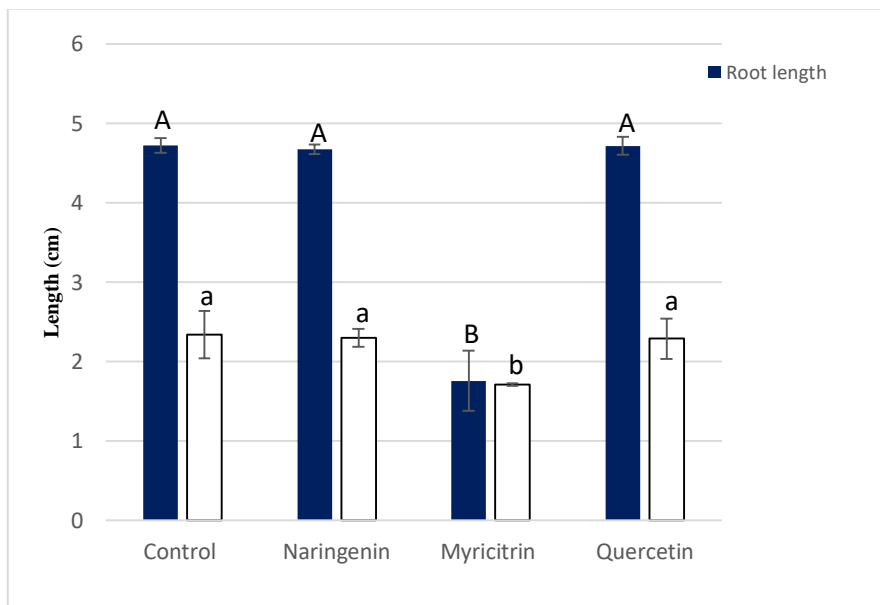


Figure 30 : Pre-emergence activity of myricitrin at 60 $\mu\text{g/ml}$, quercetin at 250 $\mu\text{g/ml}$ and naringenin at 100 $\mu\text{g/ml}$ on *T. incarnatum* after 5 days. Values in a column followed by the same letter are not significantly different at $P < 0.05$, as established by Tukey's test.

4. Discussion

In our study, we have evaluated the herbicidal effect of *C. cardunculus* crude extract on *T. incarnatum* under laboratory conditions. It varied significantly according to the crude extract concentration, the maximal effect being obtained with the highest concentration, which is often the case in the literature [20 ; 21]. The apparition of secondary roots for the treated seeds in pre-emergence tests could be caused by the interaction of the phenolic compounds from our extract with auxin transport [14] which affects the cell division and elongation processes during seed germination [22]. In addition, they could interact with enzymes involved in the mobilization of nutrients necessary for germination [23].

We have also studied the herbicidal effect of *C. cardunculus* crude extract in post-emergence, under greenhouse conditions by measuring physiological and biochemical parameters of treated *T. incarnatum*. The leaves showed signs of injury represented by several necrosis and chloroses, from 3 days after spraying on *T. incarnatum* leaves. These damages resulted in the increase of dry matter and of fluorescence observed in the leaves treated by the formulated extract. The fluorescence measurement has already been used in several studies to investigate the physiological aspects of photosynthesis and characterize plant photosynthetic performance [16]. For example, the study of [24] showed that *Eucalyptus globulus* aqueous extract affected the photochemical status of the treated plant. Indeed, the phenolic compounds in that extract (ferulic and p-hydroxybenzoic acids) decreased the chlorophyll fluorescence efficiency, represented by the ratio of variable to maximum fluorescence the Fv/Fm. This phenomenon was proven to be related to an inhibition of osmotically driven uptake of water under oxidative stress leading to a water content decrease and an increase in the percentage of the dry matter. In addition, we have confirmed that the formulation significantly improves the herbicidal effects of the extract. As described by [9], the formulation containing a vegetable oil and nonionic surfactants increases their adsorption rate of active compounds, dissolves cuticular fatty acids and therefore improves the penetration of the hydrophilic active substances [9; 24; 25].

The symptoms observed in our study could be linked to the formation of chlorophyllases (responsible of chlorophyll degradation and the change of thylakoid membrane structure) like it was observed by [26] studying the herbicidal effect of *Cupressus sempervirens*, *Juniperus phoenicea* and *Tetraclinis articulata* extracts. Another possibility is that an oxidative stress caused an excess of energy, transferred to oxygen via chlorophyll and resulting in photo-oxidation damage. Excessive damage leads to membranes destruction and chlorophyll oxidation [27]. The measurement of the biochemical parameters (increase of MDA, H₂O₂ production and lipid peroxidation levels) of treated *T. incarnatum* showed that our extract causes an oxidative stress that could initiate a sequence of reactions inducing damages cellular organelles, ultimately leading to cell death [17]. For example, the H₂O₂ produced in mitochondria, can react with the iron content to generate more reactive radicals like OH·, damaging mitochondria and leading to ROS accumulation in the cells [17]. Lipid peroxidation originating

from an oxidative degradation of the membrane lipids, produces MDA in the various cell organelles and can therefore disrupt membrane integrity, induce oxidative phosphorylation and inhibit the electron transfer chain [28]. Moreover, the high production of H₂O₂ could interfere with the activity of enzymes containing -SH groups, and therefore inhibit photosynthetic activity [29].

Several studies have already shown that phenolic compounds have phytotoxic effects and could affect the cellular metabolism of the plant. Sakihama *et al.*, 2002 showed that they may induce the formation of ROS and consequently produce pro-oxidant compounds like phenoxy groups that could be detoxified by enzymatic and non-enzymatic reactions. However, under certain conditions (pH change, presence of ions such as Al, Zn, Mg, Cu, Fe, Cd or Ca), these phenoxy radicals can react with oxygen to generate H₂O₂, the hydroxyl radical (OH.), and a complex combination of semiquinones and quinones. The hydroxyl radical is known as the strongest oxidant and may cause enzyme inactivation, protein degradation, DNA damage, lipid peroxidation and ultimately cell death [30 ; 31].

On the other hand, the conductivity was increased in treated leaves of *T.incarnatum* in comparison to the control. It has been previously described by [32] that necrosis observed in treated leaves might be due to the leakage of ions and metabolites. This can be related to an alteration of the cell membrane and consequently cell death. On the other hand, [33; 34] have observed that flavonoids could inhibit electron transport in Photosystem II (PSII) and reduce the enzymatic activity of plastoquinone which is an important coenzyme in the electron transfer chain during photosynthesis. They strongly altered and degraded chloroplast ultrastructure of leaf parenchyma [35]. [36] confirmed that the high bioactivity of flavonoids can be linked to their ability to interact with membranes. It has also been shown that naringenin can have a pro-oxidant effect on the lipids of membrane and could even cause cleavage of cell DNA via the production of phenoxy radicals by forming complexes with transition metals [37]. They could hence damage cell membrane and alter metabolic functions [27]. The plasma membrane delimits the cell from its environment and has a fundamental function in perceiving the signals received from the outside to provide exchanges between the cytoplasm and the cellular environment [38 ; 39]. As a consequence, any modification of the plant plasma membrane (PPM) structure by bioactive molecules will disturb its function

and integrity and hence affect the biochemical and physiological processes of the cell [40].

In that view, the phytotoxic effects that we observed could be linked to the fact that PPM is one of the targets of the individual compounds from the crude extract, due to their lipophilicity and their small size, as shown for some essential oils compounds [41]. To test this hypothesis, we used the “*in silico*” IMPALA method to predict the ability of molecules to penetrate into a model membrane (Figure 8). Quercetin does not penetrate into the membrane while naringenin and myricitrin could interact with the polar part of the membrane but seem to not be able to cross the apolar domain of the membrane. Since naringenin and myricitrin are more phytotoxic than quercetin, we can assume that the PPM could be involved in their biological activity, as shown for other natural compounds, such as gramin or hordenin [42].

In our study, we have also looked at the combined effects of the main compounds of our crude extract. It was already shown by [9] that myricitrin, naringenin and quercetin tested alone were phytotoxic compounds, but no study was presenting an herbicidal effect of mixed phenolic compounds to our best knowledge. We have hence observed that naringenin increases the herbicidal effect of myricitrin. In line with these observations, [4] showed that two amine alkaloids, sarmentosine and sarmentine isolated from *Piper sarmentosum* have a high potential as a bioherbicide. Similarly, the mixtures of xanthoxyline and other natural compounds (alkene, phenolic aldehyde and unsaturated fatty acid) in a Tween® 80 solution have been proven to be able to significantly inhibit the germination and root growth of Chinese amaranth at low concentrations [42]. They confirmed that the synergistic effects of these compounds could be applied for the development of potential herbicides with lower risk of resistance in weeds.

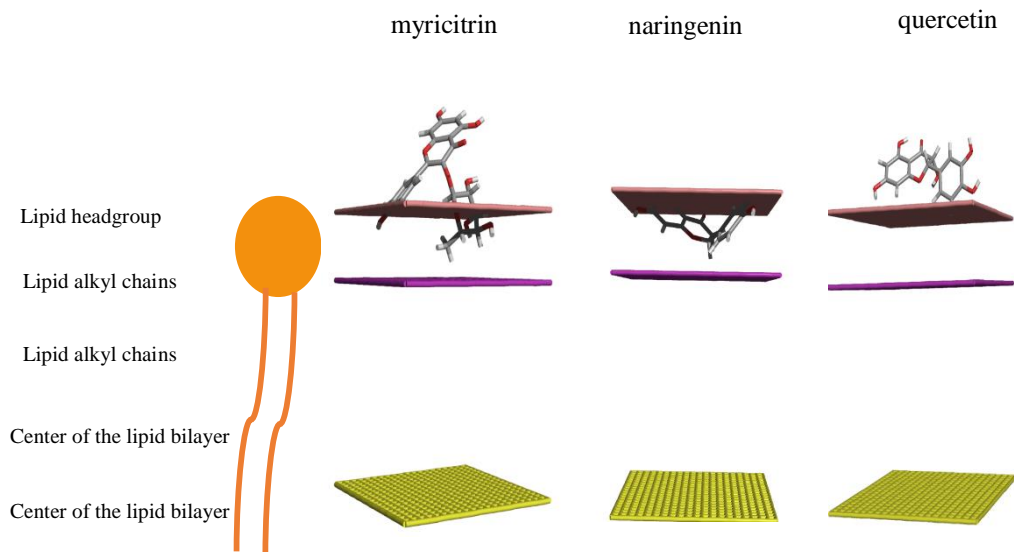


Figure 31 : 3D representation of the optimal energy position for the interaction of flavonoids calculated by the IMPALA method. The pink line represents the outer medium /membrane interface, the mauve surface is the limit between the hydrophilic heads and the hydrophobic tails of the lipids and the yellow surface represents the centre of the membrane.

5. Conclusion

Understanding the correlation between the mode of action of *C. cardunculus* crude extract and the oxidative stress seems to be one of the most challenging aspects of this study. The use of adjuvants could be a key role in the mode of action of our formulated extract. We can assume that the insertion of myricitrin and naringenin into the plasma membrane could be one of the main signals for electrolyte leakage and production of high levels of phenoxyl radicals. Moreover, the electrolyte leakage causes directly a disturbance in the electron transport chain in photosynthetic system, consequently increases ROS production, and decreases ATP levels (a source of energy in the cell). In this case, ROS can deactivate proteins, stop the cell division, and therefore cause cell death. All these modifications can cause many physiological processes that are observed in plant treated leaves. Among these modifications, we have noticed chloroses, necrosis, high percentage of dry weight and the decrease of fluorescence. A hypothetical scheme for the mode of action for our phenolic compounds in plants is proposed in Figure 32.

On the other hand, our experiments shed some light on the effects of *C. cardunculus* individual bioactive components. An optimal formulation containing myricitrin and naringenin and having other modes of action than conventional herbicides, notably by potentially targeting the plasma membrane, could be highly useful to solve the herbicide resistance issue. Overall, *C. cardunculus* crude extract can be suggested as a potential eco-friendly herbicide and suitable source of natural herbicidal compounds.

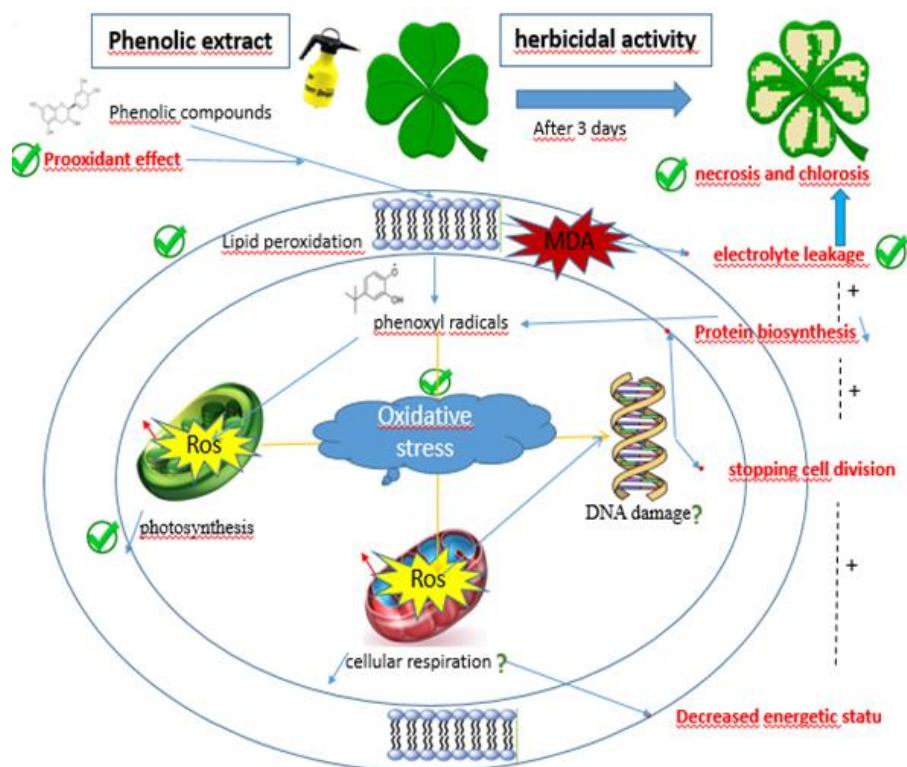


Figure 32: A hypothetical scheme for herbicidal effect mediated by phenolic compounds. Interaction of the phenolic compounds with the polar part of the membrane shown induces a prooxidant effect by forming phenoxyl radicals which are very toxic for the cell. Phenoxyl radicals reduced photosynthetic activity by overproduction of the ROS.

Author Contributions: All authors have read and agree to the published version of the manuscript. Conceptualization, Sofiene Ben kaab. and Laurence Lins; methodology, Sofiene Ben kaab and Magali Deleu; software, Laurence Lins; validation, Marie-Laure Fauconnier; Haissam Jijakli ; Riadh ksouri and Caroline De Clerck writing—original draft preparation, Marwa Hanafi; Ines Bettaieb writing—review and editing, Sofiene Ben kaab and Caroline De Clerck.; visualization, Laurence Lins; Magali Deleu and Haissam Jijakli.; supervision Haissam Jijakli; Riadh Ksouri and Caroline De Clerck; project

administration Haissam Jjakli and Riadh Ksouri.; funding acquisition, Haissam Jjakli and Riadh Ksouri.

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References

1. Van Evert, F.K.; Fountas, S.; Jakovetic, D.; Crnojevic, V.; Travlos, I.; Kempenaar, C. Big Data for weed control and crop protection. *Weed Res.* **2017**, *57*, 218–233.
2. Araniti, F.; Mancuso, R.; Lupini, A.; Giofrè, S.; Sunseri, F.; Gabriele, B.; Abenavoli, M. Phytotoxic Potential and Biological Activity of Three Synthetic Coumarin Derivatives as New Natural-Like Herbicides. *Molecules* **2015**, *20*, 17883–17902.
3. Soltys, D.; Krasuska, U.; Bogatek, R.; Gniazdowska, a. Allelochemicals as Bioherbicides - Present and Perspectives. *Herbic. - Curr. Res. Case Stud. Use* **2013**, 517–542.
4. Feng, G.; Chen, M.; Ye, H.C.; Zhang, Z.K.; Li, H.; Chen, L.L.; Chen, X.L.; Yan, C.; Zhang, J. Herbicidal activities of compounds isolated from the medicinal plant *Piper sarmentosum*. *Ind. Crops Prod.* **2019**, *132*, 41–47.
5. Dayan, F.E.; Owens, D.K.; Duke, S.O. Rationale for a natural products approach to herbicide discovery. *Pest Manag. Sci.* **2012**, *68*, 519–528.
6. Ksouri, R.; Ksouri, W.M.; Jallali, I.; Debez, A.; Hiroko, I.; Abdelly, C. Medicinal halophytes : potent source of health promoting biomolecules with medical , nutraceutical and food applications. *Crit Rev. Biotech.* **2012**, *32*(4) : 289-326.
7. Ben Kaab, S.; Rebey, I.B.; Hanafi, M.; Berhal, C.; Fauconnier, M.L.; De Clerck, C.; Ksouri, R.; Jijakli, H. *Rosmarinus officinalis* essential oil as an effective antifungal and herbicidal agent. *Spanish J. Agric. Res.* **2019**, *17*, e1006.
8. Bessada, S.M.F.; Barreira, J.C.M.; Oliveira, M.B.P.P. Asteraceae species with most prominent bioactivity and their potential applications: A review. *Ind. Crops Prod.* **2015**, *76*, 604–615.
9. Ben kaab, S.; Rebey, I.B.; Hanafi, M.; Hammi, K.M.; Smaoui, A.; Fauconnier, M.L.; De Clerck, C.; Jijakli, M.H.; Ksouri, R. Screening of Tunisian plant extracts for herbicidal activity and formulation of a

- bioherbicide based on *C. cardunculus*. *South African J. Bot.* **2020**, *128*, 67–76.
10. Kagan, V.E.; Serbinova, E.A.; Bakalova, R.A.; Stoytchev, T.S.; Erin, A.N.; Prilipko, L.L.; Evstigneeva, R.P. Mechanisms of stabilization of biomembranes by alpha-tocopherol. The role of the hydrocarbon chain in the inhibition of lipid peroxidation. *Biochem. Pharmacol.* **1990**, *40*, 2403–2413.
 11. Chuah, T.S.; Ismail, B.S. Evaluation of the biochemical and physiological activity of the natural compound , 2 , 4-ditert-butylphenol on weeds. *Crop Past Science* **2015**, 214–223.
 12. Weir, T.L.; Park, S.-W.; Vivanco, J.M. Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Opin. Plant Biol.* **2004**, *7*, 472–479.
 13. Hazrati, H.; Saharkhiz, M.J.; Niakousari, M.; Moein, M. Natural herbicide activity of *Satureja hortensis* L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. *Ecotoxicol. Environ. Saf.* **2017**, *142*, 423–430.
 14. Ribeiro, R.C.; Feitoza, R.B.B.; Lima, H.R.P.; Geraldo De Carvalho, M. Phytotoxic effects of phenolic compounds on *Calopogonium mucunoides* (Fabaceae) roots. *Aust. J. Bot.* **2015**, *63*, 679–686.
 15. Lim, C.J.; Basri, M.; Ee, G.C.L.; Omar, D. Phytoinhibitory activities and extraction optimization of potent invasive plants as eco-friendly weed suppressant against *Echinochloa colona* (L.) Link. *Ind. Crops Prod.* **2017**, *100*, 19–34.
 16. Digrado, A.; De la Motte, L.G.; Bachy, A.; Mozaffar, A.; Schoon, N.; Bussoti, F.; Amelynck, C.; Dalcq, A.C.; Fauconnier, M.L.; Aubinet, M.; Heinesh, B.; Du jardin, P.; Delaplace, P. Decrease in the photosynthetic performance of temperate grassland species Does not lead to a decline in the Gross primary production of the Ecosystem. *Front. Plant Sci.* **2018**, *9*.
 17. Kaur, S.; Singh, H.P.; Batish, D.R.; Kohli, R.K. *Artemisia scoparia*

- essential oil inhibited root growth involves reactive oxygen species (ROS)-mediated disruption of oxidative metabolism: In vivo ROS detection and alterations in antioxidant enzymes. *Biochem. Syst. Ecol.* **2012**, *44*, 390–399.
18. Lins, L.; Brasseur, R.; Malaisse, W.J.; Biesemans, M.; Verheyden, P.; Willem, R. Importance of the hydrophobic energy: Structural determination of a hypoglycemic drug of the meglitinide family by nuclear magnetic resonance and molecular modeling. *Biochem. Pharmacol.* **1996**, *52*, 1155–1168.
 19. Ducarme, P.; Rahman, M.; Brasseur, R. Impala: A simple constraint field to simulate the biological membrane in molecular structure studies. *J. Pharm. Belg.* **1998**, *53*, 106.
 20. Jelassi, A.; Ayeb-Zakhama, A. El; Nejma, A. Ben; Chaari, A.; Harzallah-Skhiri, F.; Jannet, H. Ben Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species. *Ind. Crops. Prod.* **2016**, *83*, 339–345.
 21. Omezzine, F.; Ladhari, A.; Rinez, A.; Haouala, R. Potent herbicidal activity of *Inula crithmoïdes* L. *Sci. Hortic.* **2011**, *130*, 853–861.
 22. Yan, Z.; Wang, D.; Cui, H.; Zhang, D. Phytotoxicity mechanisms of two coumarin allelochemicals from *Stellera chamaejasme* in lettuce seedlings. *Acta. Physiol. Plant* **2016**, *38* : 248.
 23. Kamal, J. Impact of allelopathy of sunflower (*Helianthus annuus* L .) roots extract on physiology of wheat (*Triticum aestivum* L). *Afric.J. Biotech* **2011**, *10(65)*, 14465–14477.
 24. Puig, C.G.; Reigosa, M.J.; Valentão, P.; Andrade, P.B.; Pedrol, N. Unravelling the bioherbicide potential of *Eucalyptus globulus* Labill: Biochemistry and effects of its aqueous extract. *PLoS One* **2018**, *13*, 1–16.
 25. Todero, I.; Confortin, T.C.; Luft, L.; Brun, T.; Ugalde, G.A.; de Almeida, T.C.; Arnemann, J.A.; Zobot, G.L.; Mazutti, M.A. Formulation of a bioherbicide with metabolites from *Phoma* sp. *Sci. Hortic.* **2018**, *241*, 285–292.

26. M'barek, K.; Zribi, I.; Ullah, M.J.; Haouala, R. The mode of action of allelochemicals aqueous leaf extracts of some Cupressaceae species on lettuce. *Sci. Hortic.* **2019**, *252*, 29–37.
27. Mahdaviakia, F.; Saharkhiz, M.J.; Karami, A. Defensive response of radish seedlings to the oxidative stress arising from phenolic compounds in the extract of peppermint (*Mentha × piperita* L.). *Sci. Hortic.* **2017**.
28. Ben Ghnaya, A.; Hanana, M.; Amri, I.; Balti, H.; Gargouri, S.; Jamoussi, B.; Hamrouni, L. Chemical composition of *Eucalyptus erythrocorys* essential oils and evaluation of their herbicidal and antifungal activities. *J. Pest Sci. (2004)*. **2013**, *86*, 571–577.
29. Harminder, P.; Batish, D.R.; Kaur, S.; Arora, K.; Kohli, R.K. α - Pinene Inhibits Growth and Induces Oxidative Stress in Roots. *Annals Bot.* **2006**, *98*, 1261–1269.
30. Sakihama, Y.; Cohen, M.F.; Grace, S.C.; Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. *Toxicology* **2002**, *177*, 67–80.
31. Eghbaliferiz, S.; Iranshahi, M. Prooxidant Activity of Polyphenols , Flavonoids , Anthocyanins and Carotenoids : Updated Review of Mechanisms and Catalyzing Metals. **2016**, *1391*, 1379–1391.
32. Hejl, A.M.; Koster, K.L. juglone disrupts root plasma membrane h - atpase activity and impairs water uptake , root respiration , and growth in soybean (*glycine max*) and corn (*zea mays*). *J. Chem. Eco***2004**, *30*, 453–471.
33. Liu, M.; Lu, S. Plastoquinone and Ubiquinone in Plants: Biosynthesis, Physiological Function and Metabolic Engineering. *Front. Plant Sci.* **2016**, *7*, 1–18.
34. Tigre, R.C.; Pereira, E.C.; da Silva, N.H.; Vicente, C.; Legaz, M.E. Potential phenolic bioherbicides from *Cladonia verticillaris* produce ultrastructural changes in *Lactuca sativa* seedlings. *South African J. Bot.* **2015**, *98*, 16–25.

35. Tsuchiya, H. Structure-dependent membrane interaction of flavonoids associated with their bioactivity. *Food Chem.* **2010**, *120*, 1089–1096.
36. Tarahovsky, Y.S.; Kim, Y.A.; Yagolnik, E.A.; Muzafarov, E.N. Flavonoid-membrane interactions: Involvement of flavonoid-metal complexes in raft signaling. *Biochim. Biophys. Acta - Biomembr.* **2014**, *1838*, 1235–1246.
37. Stillwell, W.; *Book*, Introduction to Biological Membranes 2nd ed.; *Elsevier* **2016**, *3*, 154–196.
38. Erlejman, A.G.; Verstraeten, S. V.; Fraga, C.G.; Oteiza, P.I. The interaction of flavonoids with membranes: Potential determinant of flavonoid antioxidant effects. *Free Radic. Res.* **2004**, *38*, 1311–1320.
39. Deleu, M.; Crowet, J.; Nasir, M.N.; Lins, L. Complementary biophysical tools to investigate lipid specificity in the interaction between bioactive molecules and the plasma membrane: A review. *Bioch Bioph Acta.* **2014**, 3171-3190
40. Lins, L.; Dal Maso, S.; Foncoux, B.; Kamili, A.; Laurin, Y.; Genva, M.; Jijakli, M.H.; De Clerck, C.; Fauconnier, M.L.; Deleu, M. Insights into the Relationships Between Herbicide Activities, Molecular Structure and Membrane Interaction of Cinnamon and Citronella Essential Oils Components. *Int. J. Mol. Sci.* **2019**, *20*, 4007.
41. Lebecque, S.; Crowet, J.; Lins, L.; Delory, B.M.; Patrick, J.; Fauconnier, M.; Deleu, M. Interaction between the barley allelochemical compounds gramine and hordenine and artificial lipid bilayers mimicking the plant plasma membrane. *Front. Plant. Sci* **2018**, 1–13.
42. Chotsaeng, N.; Laosinwattana, C.; Charoenying, P. Herbicidal activities of some allelochemicals and their synergistic behaviors toward *amaranthus tricolor* L. *Molecules* **2017**, *22*, 1–15.

5

Valorisation des résultats

Le caractère innovant et original de cette thèse a été étudié. En effet, même si les études sont moins nombreuses, certains composés phénoliques et en particulier les flavonoïdes sont connus pour leurs propriétés herbicides mais aucune publication n'a été réalisée sur l'activité herbicide de la myricitrine et aucune étude pertinente n'a été réalisée afin d'élaborer une formulation d'un bioherbicide à base d'extraits végétaux et étudier par la suite son spectre d'action vis à vis d'une série d'adventices problématiques Ceci a été valorisé par la publication d'un brevet d'invention international (PCT). Ce brevet d'invention protège toute composition herbicide à base d'un ou plusieurs composés phénoliques. Cette composition contient une substance active à partir d'une plante spontanée tunisienne (y compris *C. cardunculus*), de l'huile végétale (inclu huile végétale de noisette), des surfactants non ioniques et un solvant organique (inclu l'éthanol)

La méthodologie utilisée ainsi que tous les essais biologiques sont décrites dans les différents articles de cette thèse. Par contre, les concentrations et les résultats de l'optimisation de la formulation restent jusqu'à présent confidentiels.

Herbicidal composition comprising at least one phenolic active compound

Field of the invention

The invention relates to a herbicidal composition comprising at least one phenolic active compound. The invention also relates to the use of such a herbicidal composition.

Description of prior art

Weeds have been documented as serious plant pests, which can reduce crop yields and interfere with plant functions to stop their growth. They constantly compete with crops for water and nutrient resources reducing yields and quality and, consequently, causing huge economic losses which can reach 34% in major crops.

Currently, most reliable weed control methods include mechanical weeding, hand weeding and application of chemical herbicides. These last chemical methods pose several problems with their negative impacts on environmental, animal and human health. Also, the systematic application of chemical herbicides increases the weed's resistance to the applied chemical molecules/substances.

The current trend is to find a biological product to minimize the perceived impacts from synthetic herbicides in agriculture production. In this sense, allelopathy can be importantly used to identify new natural compounds and to discover new herbicide target sites. This is the reason why allelopathy can be added to the current repertoire of commercial herbicide mode of action. More particularly, allelopathy is a biochemical interaction of inhibition and promotion within plants or microorganisms through the release of allelochemicals into the environment via root exudation, volatile emissions, leaching from leaves, other aerial parts, and decomposition of plant material. It plays a major role in agro- ecosystems, such as weed control, crop protection, due to its adverse effects on germination and seedling growth. Allelochemicals, especially those that cause damage to receptor plants, have drawn increasing attention in the development of promising alternatives to conventional herbicides in crop protection. Allelochemicals were derived from crop plants which are generally secondary metabolites as phenolic

compounds, terpenoids, alkaloids, coumarins, tannins, flavonoids, steroids and quinones.

Phenolic compounds are one of the most categories of the secondary metabolites implicated in plant allelopathy. They represent one of the largest groups, more investigated in relation to their ecological role in plant–soil system and in plant–plant interactions. They are synthesized via the phenylpropanoid pathway and play a role in plant defense mechanisms against biotic and abiotic stresses. They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes. These molecules are widely distributed in leaves, stems, seeds and fruits of many edible plants. Phenolic compounds also constitute one of the most numerous and widely distributed groups of antioxidant substances in the plant kingdom with more than 8000 phenolic structures currently known. It was proved that phenolic compounds play an important role to neutralize free radicals, and quenched singlet oxygen, or decomposing peroxides, which are inevitably produced when aerobic or photosynthetic metabolism is impaired by environmental stresses. It was also proved that these compounds are mainly produced by plants for protection against, insects, viruses and bacteria.

Some herbicidal compositions comprising phenolic compounds were notably tested in a study performed by De Martino et al. (In Vitro Phytotoxicity and Antioxidant Activity of Selected Flavonoids, *Int. J. Mol. Sci.*, 2012, 13, 5406-5419). In this study, 27 flavonoids were tested for their potential herbicidal properties on radical elongation and germination of weeds (*R. sativus* and *L. sativum*). The obtained results indicate that only four flavonoids act on germination and on radical elongation: 3',4'-di-OCH₃-flavone, 3-OCH₃-flavone, 3,5,7,3',4'-penta-OH-flavone (quercetin) and 5,7-di-OH-flavanone (pinocembrin), with only poor results on radical elongation for the highest tested concentration of 10⁻⁴M.

Thus, nowadays, only few phenolic compounds are identified for their real herbicidal properties and the compositions comprising such compounds are moderately effective both on radical elongation and germination of weeds.

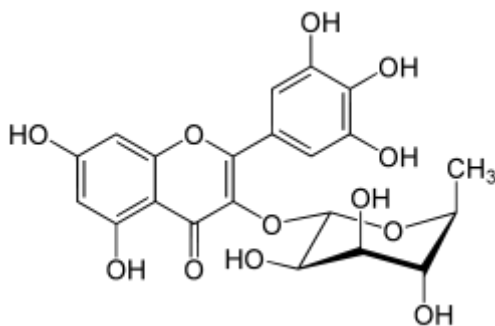
Summary of the invention

It is an object of the invention to provide a herbicidal composition comprising at least one phenolic active compound so as to be effective both on radical elongation and germination (pre-emergence herbicidal activity) of weeds but also on germinated and developed weeds (post-emergence herbicidal activity).

More particularly, it is an object of the invention to provide a herbicidal composition comprising at least one newly identified phenolic active compound as an efficient alternative to conventional herbicides in crop protection but also as an alternative to the currently known phenolic compounds used as herbicides, said one newly identified phenolic active compound presenting both a pre-emergence herbicidal activity and a post-emergence herbicidal activity.

To this end, according to the invention, there is provided a herbicidal composition comprising at least one phenolic active compound, said at least one phenolic active compound being myricitrin.

Myricitrin is a phenolic compound presenting the following chemical structure:



Compositions comprising myricitrin are known from the following documents: WO2013/184884, Laura De Martino et al. (Phytotoxic effect of bioactive compounds isolated from *Myrcia tomentosa* (Myrtaceae) leaves, *Biochemical Systematics and Ecology*, vol. 46, 1, February 2013, pages 29-35), Sofiane Dairi et al. (Antioxidant effects of extra virgin olive oil enriched by myrtle phenolic extracts on iron-mediated lipid peroxidation under intestinal conditions model, *Food Chemistry*, vol. 237, April 2017, pages 297-

304), Cheryl A. Hobbs et al. (Genotoxicity evaluation of the flavonoid, myricitrin, and its aglycone, myricetin, Food and Chemical Toxicology, vol. 83, September 2015, pages 283-292), Zelia Velez et al. (Biological characterization of *C. cardunculus* L. methanolic extracts: antioxidant, anti-proliferative, anti-migratory and anti-angiogenic activities, Agriculture, vol. 2, no. 4, December 2012, pages 472-492) and Ricardo Salazar-Aranda et al. (Activity of polyphenolic compounds against *Candida glabrata*, Molecules, vol. 20, no. 10, September 2015, pages 17903-17912).

The terms “active compound” mean that the compound is biologically active, more particularly that the compound is active as a herbicide (herbicidal activity). The desired herbicidal activity may be the sort to inhibit seed germination, to prevent the resumption of underground or aerial meristem and bud development, or to inhibit the development and growth of the roots and also of the hypocotyls, the epicotyls and any aerial parts of the plants.

The invention is defined by the independent claims. The dependent claims define advantageous embodiments.

In the context of the present invention, it was surprisingly determined that a composition according to the invention comprising myricitrin as a phenolic active compound demonstrates an efficient herbicidal activity on weeds. Indeed, it was shown that a composition according to the invention comprising myricitrin as a phenolic active compound exhibits both a pre-emergence herbicidal activity and a post-emergence herbicidal activity.

Preferably, in a herbicidal composition according to the invention, myricitrin is from synthetic origin.

Alternatively, in a herbicidal composition according to the invention, myricitrin is from an extract or from a fraction of an extract from the roots, the leaves, the stems, the fruits, the seeds and/or the flowers of at least one plant chosen from the group consisting of *Limoniastrum guyonianum*, *Peganum harmala*, *Ruta chalepensis*, *Ricinus communis*, *Nitraria retusa*, *Cynara cardunculus*, *Artemisia herba-alba*, *Mesembryanthemum edule*, *Tamarix gallica*, *Datura stramonium*, *Myrica esculenta*, *Myrica cerifera*, *Nymphaea lotus*, *Nymphaea odorata*, *Chrysobalanus icaco*, *Polygonum aviculare* and mixtures thereof. It is

understood that any other plant from which an extract comprising myricitin and/or a fraction of an extract comprising myricitin can be obtained is part of the present invention. The previous list of plants is not exhaustive.

The extract used in the context of the invention can be a cellular extract or an extracellular extract of the plant, which can be prepared according to any method known to those skilled in the art for extracting compounds from plant tissues. The extract can be obtained from the roots, the leaves, the stems, the fruits, the seeds and/or the flowers.

Advantageously, the extract is derived from the leaves, the fruits or the seeds, the use of which does not lead to the death of the plant from which they are extracted.

By way of examples of extraction methods, mention may be made of the conventional methods of hot solvent extraction or cold solvent extraction, maceration, lixiviation, cryoextraction, digestion, decoction, extractions with supercritical CO₂ or extractions using undulator radiation, such as microwaves or ultrasound. These methods can be combined with a subsequent purifying, filtering, concentrating and/or drying step.

Advantageously, the extract used is obtained by extraction with a solvent selected from water, alcohols, alkanes, ketones, esters, ethers, polyols, chlorinated solvents and mixtures thereof. In particular, extraction may be carried out using a solvent, such as an alcoholic solvent selected from ethanol or methanol or else polyethylene glycol or chloroform.

The extract obtained can then be concentrated, or dried, for example by evaporation or lyophilization. The extract obtained can be incorporated as it is into the composition or else be purified, treated or fractionated, so as to enrich for desired active ingredient.

Fractionation can be for example performed by centrifugal partition chromatography or by reverse phase.

Advantageously, in a herbicidal composition according to the invention, myricitrin is present at a concentration ranging from 0.1 mg/L to 10000 mg/L, preferably ranging from 0.25 mg/L to 5000 mg/L, more preferably ranging from 0.5 mg/L to 2500 mg/L, even more preferably ranging

from 0.75 mg/L to 1500 mg/L, even more preferably ranging from 0.8 mg/L to 500 mg/L, even more preferably ranging from 1 mg/L to 100 mg/L.

Preferably, a herbicidal composition according to the invention further comprises at least one additional active compound chosen from the group consisting of syringic acid, p-coumaric acid, quercetin, naringenin, catechin, apigenin, gallic acid, cynarin acid, chlorogenic acid, cynaroside, luteolin, aguerin B, grosheimin, cynaropicrin and mixtures thereof. It is understood that any other appropriate additional active compound is part of the present invention. The previous list of plants is not exhaustive.

Advantageously, in a herbicidal composition according to the invention, said at least one additional active compound is from synthetic origin.

More advantageously, in a herbicidal composition according to the invention, said at least one additional active compound is from an extract or from a fraction of an extract from the roots, the leaves, the stems, the fruits, the seeds and/or the flowers of at least one plant chosen from the group consisting of *Limoniastrum guyonianum*, *Peganum harmala*, *Ruta chalepensis*, *Ricinus communis*, *Nitraria retusa*, *Cynara cardunculus*, *Artemisia herba-alba*, *Mesembryanthemum edule*, *Tamarix gallica*, *Datura stramonium*, *Myrica esculenta*, *Myrica cerifera*, *Nymphaea lotus*, *Nymphaea odorata*, *Chrysobalanus icaco*, *Polygonum aviculare* and mixtures thereof. It is understood that any other plant from which said at least one additional active compound can be obtained is part of the present invention. The previous list of plants is not exhaustive.

Preferably, in a herbicidal composition according to the invention, said at least one additional active compound is present at a concentration ranging from 0.1 mg/L to 10000 mg/L, preferably ranging from 0.2 mg/L to 5000 mg/L, more preferably ranging from 0.25 mg/L to 500 mg/L.

Preferably, a herbicidal composition according to the invention further comprises at least one solvent chosen from the group consisting of at least one natural lipophilic solvent, at least one synthetic lipophilic solvent, at least one natural hydrophilic solvent, at least one synthetic hydrophilic solvent, at least one natural amphiphilic solvent, at least one synthetic amphiphilic solvent and mixtures thereof.

Preferably, in a herbicidal composition according to the invention, said at least one lipophilic solvent is chosen from the group consisting of animal oil, vegetable oil, petrochemical oil and mixtures thereof.

For example, according to the invention, said vegetable oil is chosen from the group consisting of hazelnut oil, rapeseed oil, pine oil, soybean oil, sunflower oil, pistachio oil, olive oil, almond oil, sweet almond oil, peanut oil and mixtures thereof.

Preferably, in a herbicidal composition according to the invention, said at least one hydrophilic solvent is chosen from the group consisting of water, alcohols, ketones, esters, ethers, polyols, ethyl acetate, propylene carbonate, acetonitrile and mixtures thereof.

Advantageously, in a herbicidal composition according to the invention, said at least one natural amphiphilic solvent and/or said at least one synthetic amphiphilic solvent is at least one non-ionic surfactant. This means that, according to the present invention, a non-ionic surfactant can play the role of a solvent. For example, Tween 20® can be considered as a synthetic amphiphilic solvent.

Non-ionic surfactants are amphiphilic substances and have covalently bonded oxygen-containing hydrophilic groups, which are bonded to hydrophobic parent: *non-ionic surfactants* consist of a hydrophilic head group and a hydrophobic tail.

Preferably, according to the present invention, said at least one non-ionic surfactant is chosen from the group consisting of sorbitan monododecanoate poly(oxy-1,2-ethanediyl), alkyl polyglucosides, sorbitan laurate, polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (20) sorbitan monopalmitate, polyoxyethylene (20) sorbitan monostearate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene glycol sorbitan laurate, hexaethylene glycol sorbitan monooleate, polyoxyethylene sorbitan stearate, decaglyceryl monooleate, decaglyceryl dioleate, polyoxyethylene sorbitan tristearate, monodehydrosorbitol monooleate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan laurate and mixtures thereof. It is understood that any other appropriate surfactant is part of the present invention. The previous list of plants is not exhaustive.

Commercial names of adequate non-ionic surfactants are for example: Tween 20®, Tween 80®, Span 80®, Atplus® UEP-100, ethoxylated castor oil, Span 85®, Brij® 93, Brij® S 100, IGEPAL® CA-210, IGEPAL® CO-890, MERPOL® HCS, SP Brij® C2 MBAL-SO-(SG), Triton™ x-405 reduced, Etocas® 5, glycerol monostearate, glyceryl stearate, Emulson® CO 9, Span 20® et Span 80® and mixtures thereof.

Advantageously, according to the present invention, said at least one non-ionic surfactant presents a hydrophilic-lipophilic balance (HLB) value comprised between 5 and 7, preferably a HLB value equal to 6.

Such a HLB value of said at least one non-ionic surfactant comprised between 5 and 7 and more preferably equal to 6 was determined, in the context of the present invention, to be the adequate HLB value in order to obtain a composition under the form of an emulsion with the right droplet size allowing the composition to effectively penetrate the plant membranes. Preferably, according to the present invention, the composition under the form of an emulsion presents droplets having a droplet size comprised between 0.05 µm et 10 µm, preferably a droplet size comprised between 0.1 µm and 2 µm, more preferably a droplet size comprised between 0.2 µm and 1 µm.

It was also shown that the compositions according to the invention presenting such a HLB value were more effective in term of pre-emergence and post-emergence herbicidal activity. Also, it was determined that such a HLB value allows to obtain stable composition without the formations of precipitates or supernatants.

Within the meaning of the present invention, the term “HLB” is understood as being an empirical expression which expresses the hydrophilic and hydrophobic (or lipophilic) relationship of a surfactant. The hydrophilic-hydrophobic balance (HLB) of a surfactant expresses its properties: a surfactant has a greater affinity for water if the HLB balance is high (hydrophilic nature) and, conversely, a surfactant has a lower affinity for water (lipophilic or hydrophobic nature) when its HLB value is low.

The determination of the HLB value of a non-ionic surfactant by calculation allows an arbitrary scale for non-ionic polyethoxylated surfactants to be defined. The calculation method can be as follows: $HLB=20 \times Mh/M$.

This empirical formula allowing the HLB value of a non-ionic surfactant to be calculated comprises the ratio between the molecular mass of the hydrophilic group of the non-ionic surfactant in question (M_h) multiplied by 20 (molar mass of the ethoxylated group $[(-CH_2-CH_2-O-)_n]$ of the non-ionic surfactant) and the molecular mass of the surfactant in question (M).

Starting from this empirical formula, an arbitrary scale is therefore defined and has HLB values comprised between 0 and 20. An HLB value of zero corresponds to a completely lipophilic surfactant, and an HLB value of 20 corresponds to a completely hydrophilic surfactant. Non-ionic surfactants are therefore classified according to this empirically established scale. A distinction is made, therefore, between non-ionic surfactants of low, medium and high HLB: a surfactant of low HLB has an HLB value of from 0 to 6; a surfactant of medium HLB has an HLB value of from 6 to 14 and a surfactant of high HLB has an HLB value of from 14 to 20.

Finally, the HLB value of a non-ionic surfactant allows the solubility of the surfactant in water or in oil to be indicated and therefore the direction of the emulsion (water-in-oil or oil-in-water) to be determined. For this reason, a non-ionic surfactant of high HLB will have a greater affinity for water and will therefore be more soluble in water than in oil and vice versa for surfactants of low HLB, which will be more soluble in oil. A surfactant that is more soluble in water than in oil will influence the direction of the "oil-in-water" emulsion and vice versa for a surfactant that is more soluble in oil than in water.

In the sense of the present invention, said at least one non-ionic surfactant can be a mixture of non-ionic surfactants. For example, such a mixture of non-ionic surfactants can be a mixture of a low HLB non-ionic surfactant with a high HLB non-ionic surfactant. As an example, a mixture of non-ionic surfactants can be a mixture of a first surfactant having a HLB value comprised between 3 and 6 so allowing to disperse an aqueous phase in a lipophilic phase with a second surfactant having a HLB value comprised between 15 and 18 so allowing to disperse a lipophilic phase in an aqueous phase.

In the case of a binary mixture of non-ionic surfactants, the HLB value of this mixture can be calculated as followed:

binary mixture $HLB = [m_1 / (m_1 + m_2) \cdot HLB_1] + [m_2 / (m_1 + m_2) \cdot HLB_2]$

where m_1 = mass of the first surfactant

m_2 = mass of the second surfactant

HLB_1 = HLB of the first surfactant

HLB_2 = HLB of the second surfactant

According to the invention, like for compositions only comprising one surfactant having a HLB value comprised between 5 and 7, the binary mixture HLB value is preferably comprised between 5 and 7, more preferably equal to 6.

Preferably, in an herbicidal composition according to the invention, said at least one non-ionic surfactant is present in the composition at a concentration ranging from 0.1 g/L to 500 g/L, preferably ranging from 0.25 g/L to 250 g/L, more preferably ranging from 0.5 g/L to 100 g/L, even more preferably ranging from 1 g/L to 50 g/L, even more preferably ranging from 2 g/L to 25 g/L.

Advantageously, a herbicidal composition according to the invention further comprises at least one adjuvant chosen from the group consisting of paraffin wax, Heliosol[®], 1-octyl-2-pyrrolidone, Actirob[®] B, Atplus[®] 463, Actilandes[™], Vegelix[®], Emulsol[®] and mixtures thereof.

Preferably, a herbicidal composition according to the invention is under the form of granules, liquids, emulsions or concentrates. Any type of known herbicidal composition may be prepared.

The present invention is also about the use of a composition according to the invention for obtaining phytotoxic activity comprising inhibiting seed germination, preventing the resumption of underground or aerial meristem and bud development, or inhibiting the development and growth of the roots, the development and growth of the hypocotyls, the development and growth of the epicotyls, the development and growth of the plantlets or the development and growth of aerial parts of the plant. In particular, use may be made of herbicidal compositions in the form of granules, liquids, emulsions or emulsifiable concentrates, as adjuvants for herbicidal mixture.

In the context of the present invention, the term “inhibiting” means the ability of a composition to stop or slow down a biological process. For example, inhibiting seed germination means slowing down or totally suppressing the germination process and inhibiting the development and growth of the roots, the development and growth of the hypocotyls, the development and growth of the epicotyls, the development and growth of the plantlets or the development and growth of aerial parts of the plant means slowing down or totally suppressing these biological processes.

Detailed description of embodiments of the invention

Plant material, extraction and fractionation procedure

Fresh aerial parts of *Cynara cardunculus* (collected from Enfidha region in vegetative stage on February 2015 in Tunisia) were shade dried for 15 days at 30°C.

C. cardunculus extract was obtained by magnetic stirring of 10 g dry powder of the previously dried aerial parts with 100 ml pure methanol (Emplura EMD Millipore Corporation - Merck KGaA) for 30 min. Methanol was eliminated along rotavapor in vacuum at 45°C and redissolved in Tween 20® 1%. Extract was kept for 24 h at 4°C, filtered through a Whatman No 4 filter paper, and stored at 4°C until analyses. The extraction yield was 9.71 %. The methanolic filtrate was combined, concentrated under vacuum and eventually fractionated on reverse phase silica gel (Sigma-Aldrich filiale de Merck KGaA).

For the eventual fractionation of the extract, methanolic extract was loaded onto a column containing 10 g of C18 resin per g of dry extract followed by 80 ml of water to remove sugars and other polar compounds. Phenolic molecules adsorbed onto the resin were eluted with mixture methanol/water (1:4; 2:3; 3:2, 4:1; 5:0 v/v) as mobile phase. This fractionation system was based on 2 phases: a solid stationary phase which is C18 phase silica with 40-63 µm and a mobile one which is the eluent at different concentration of methanol. Firstly, the extract was inserted into the upper part of the silica gel using a vacuum pump. Based on the volume of the column and the amount of silica and of the extract added, 300 ml of water were used to remove the sugars in the extract. Afterwards, 200 ml of methanol / water with an increasing gradient (20%, 40%, 60%, 80% and 100%) were

added in the column to elute the polar compounds not retained by the silica gel and at the same time soluble in the eluent. Since the silica is hydrophobic, the non-polar compounds are retained on the silica but the polar compounds can cross the silica in order to recuperate them. Several fractions were collected, depending on the gradient methanol / water considered. For the present invention, the fraction obtained with the 40% gradient methanol / water is called "Fraction A" while the fraction obtained with the 60% gradient methanol / water is called "Fraction B". These two fractions (Fraction A and Fraction B) were purified, evaporated, filtered through a Whatman No 4 filter paper, and stored at 4°C until analyses.

HPLC analysis – identification of phenolic compounds

Identification of phenolic compounds was performed on *Cynara cardunculus* plant extracts and on fractions obtained from said extracts.

The identification of phenolic compounds was done using HPLC system (consisting of a vacuum degasser, an autosampler, and a binary pump with a maximum pressure of 400 bar; Agilent 1260, Agilent technologies, Germany) equipped with a reversed phase C18 analytical column of 4.6 x 100 mm and 3.5 µm particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200-400 nm. Column temperature was maintained at 25°C. The injected sample volume was 2 µl and the flow-rate of mobile phase was 0.4 mL/min. Mobile phase B was milli-Q water consisted of 0.1% formic acid and mobile phase A was Methanol. The optimized gradient elution was illustrated as follows: 0-5 min, 10-20% A; 5-10 min, 20-30% A; 10-15 min, 30-50% A; 15-20 min, 50-70% A; 20-25 min, 70-90% A; 25-30 min, 90-50% A; 30-35 min, return to initial conditions.

Myricitrin, quercetin, p-coumaric acid, naringenin and syringic acid were used as standards. Identification analysis was done by comparison of their retention time with those obtained from the extracts. For the quantitative analysis, a calibration curve was obtained by plotting the peak area against different concentrations for each identified compound at 280 nm: The obtained curves for all identified compounds showed a good linearity (with an average of $r^2 = 0.99$): $y = 38,976x + 4,1296$ for syringic acid; $y = 32,266x + 17,439$ for p-coumaric acid;

$Y = 6,7915x - 35,235$ for myricitrin; $y = 9,5824x - 7,4659$ for quercetin and $y = 23,691x - 88,898$ for naringenin. The amount of each compound was expressed as milligram per gram of residue.

The results obtained for an extract (Extract) and for two fractions (Fraction A and Fraction B) obtained from this extract are present in Table 15.

Table 15: Phenolic compounds identified and quantified in *Cynara cardunculus* plant extract and in two fractions obtained from this extract

Identified phenolic compounds	Retention time (min)	Molecular formula	Concentrations in mg/ g of dry residue		
			Extract	Fraction A	Fraction B
Syringic acid	17.8	$C_9H_{10}O_5$	0.108	NI*	1.308
Myricitrin	20.7	$C_{21}H_{20}O_{12}$	0.755	3.088	64.764
p-coumaric acid	20.09	$C_9H_8O_3$	0.487	NI*	0.576
Quercetin	23.7	$C_{15}H_{10}O_7$	0.383	17.427	1.430
Naringenin	24.3	$C_{15}H_{12}O_5$	0.359	4.486	NI*

*NI means that the concerned phenolic compound is not identified

Pre-emergence activity of a composition according to the invention

Experiment n°1

Seeds of *T. incarnatum* were obtained from ECOSEM industry (Belgium). Filter paper were moistened with (a) 2 ml of Tween 20® 1% solution (negative control), (b) 2 ml of a solution comprising *Cynara cardunculus* methanolic extract in Tween 20® 1% solution, (c) 2 ml of a

solution comprising Fraction A in Tween 20® 1% solution and (d) 2 ml of a solution comprising Fraction B in Tween 20® 1% solution.

Methanolic extract of *Cynara cardunculus* and its two fractions (Fraction A and Fraction B) obtained by fractionation were tested on germination and seedling growth of *Trifolium incarnatum* at a concentration of 6 g/L. Such a concentration corresponds to a 4.53 mg/L myricitrin concentration for the extract, a 18.52 mg/L myricitrin concentration for the Fraction A and to a 388.58 mg/L myricitrin concentration for the Fraction B.

12 petri dishes were prepared and randomly placed in a growth chamber at a constant temperature of 23°C, in darkness, for 5 days. The number of germinated seedlings was counted after these 5 days, and their root and hypocotyl lengths were measured.

The obtained results are presented in Figures 1 and 2. As it can be seen, the Fraction A was revealed more phytotoxic than the Fraction B and than the plant extract. In fact, Fraction A inhibited seed germination until 60% after 5 days of incubation (Figure 1). Moreover, this Fraction A completely inhibited the growth of the *Trifolium incarnatum* radicle and hypocotyl (Figure 2). Results obtained with Fraction B are also interesting since the seed germination rate and the radicle and hypocotyl lengths were significantly different from the control (Figures 1 and 2). Results obtained with the *Cynara cardunculus* extract are also significantly different from the control.

In summary, all the compositions according to the invention comprising myricitrin (Extract, Fraction A and Fraction B) demonstrate a pre-emergence herbicidal activity after 5 days.

Experiment n°2

Seeds of *T. incarnatum* were obtained from ECOSEM industry (Belgium). Filter papers were placed in petri dishes and moistened with 2 ml of methanol 1% solution for the seedling control, or with myricitrin solution at 100 mg/L in methanol 1% for the treated seedling. Methanol was identified as a solvent not interfering with the pre-emergence of plants.

Ten seeds of *Trifolium incarnatum* were then placed immediately in each petri dish and three replicates were prepared. All petri

dishes were randomly placed in a growth chamber at a temperature of $23\pm 1^\circ\text{C}$, in the dark and the hypocotyls and root lengths were measured after 7 days.

The obtained results are presented in Figure 5. As it can be seen, at 100 mg/L, myricitrin showed a significant phytotoxic effect on the pre-emergence of *Trifolium incarnatum* in comparison with the control: myricitrin inhibited the development of roots and hypocotyls.

Also, it was noted that root inhibition for seeds treated by myricitrin was manifested by a development of the slight secondary roots which can be caused by an oxidative stress.

In summary, myricitrin demonstrated a pre-emergence herbicidal activity and inhibited seed germination of *Trifolium incarnatum* compared with control what is confirmed by a reduction of the root and hypocotyl lengths after 7 incubation days.

Post-emergence activity of compositions according to the invention

A first experiment was performed to study the post-emergence activity of different compositions according to the invention. This first experiment was performed on 2-3 weeks old *Trifolium incarnatum* plants under greenhouse semi-controlled conditions (natural photoperiod supplemented with artificial light if needed with $23 \pm 2^\circ\text{C}$ according to the sunlight) and the following compositions according to the invention were tested: (a) formulated *Cynara cardunculus* plant extract at 31 g/L (corresponding to a 23.4 mg/L myricitrin concentration), (b) *Cynara cardunculus* plant extract at 31 g/L (corresponding to a 23.4 mg/L myricitrin concentration) in Tween 20® 1% solution, (c) formulated Fraction A of *Cynara cardunculus* plant extract at 20 g/L (corresponding to a 61.7 mg/L myricitrin concentration), (d) Fraction A of *Cynara cardunculus* plant extract at 20 g/L (corresponding to a 61.7 mg/L myricitrin concentration) in Tween 20® 1% solution and (e) formulated myricitrin at 60 mg/L.

When formulated, *C. cardunculus* plant extract, Fraction A or myricitrin were mixed with the following compounds as mentioned in Table 16.

Table 16 : Formulations of compositions according to the invention

	Extract %	Fraction A %	Myricitrin %
Plant extract	3.1		
Fraction		2	
Myricitrin			0.006
Vegetable oil of hazelnut	3.1	3.1	3.1
Span 80®	1.6	1.6	1.6
Tween 20®	0.4	0.4	0.4
Aplus® UEP-100	0.25	0.25	0.25
Ethanol	0.5	0.5	0.5
Water	91.05	92.15	94.14
Total	100	100	100

4. Seeds of *T. incanatum* were sown in pots and the obtained plants were watered daily. When the plants had two leaf stages, these were sprayed with pelargonic acid (positive control used as commercial bioherbicide at 31 g/L), with distilled water (negative control), with the compounds of the formulation only (see Table 2) or with one of the compositions (a) to (d) according to the invention and described above. There were eight treatments and three replicates per treatment maintained in a completely randomised manner. Five days after spraying, the treated weed plants were examined for visible injury levels in terms of wilting, necrosis and chlorosis. A percentage of efficacy was determined as followed: (number of wilted or necrotic leaves / total number of leaves) * 100.

The obtained results are presented in Figure 3. As it can be seen, all the compositions according to the invention present a post-emergence herbicidal activity. Plant extract of *Cynara cardunculus* and the obtained Fraction A from this extract presented a higher herbicidal activity when formulated compared to their efficacy without formulation. Formulated Fraction A at 20 g/L presented the same effect shown by pelargonic acid at 31 g/L. Formulated myricitrin tested at the same concentration than the one found in the Fraction A appeared also phytotoxic with 52.33 % of efficacy.

A second experiment was performed to study the post-emergence activity of different compositions according to the invention on another plant, i.e. *A. thaliana*. This second experiment was performed on 2-3 weeks old *Arabidopsis thaliana* plants under greenhouse semi-controlled conditions (continuous light; $23 \pm 2^\circ\text{C}$) and the following compositions according to the invention were tested: (a) formulated *Cynara cardunculus* plant extract at 31 g/L (corresponding to a 23.4 mg/L myricitrin concentration) and (e) formulated myricitrin at 60 mg/L.

When formulated, *Cynara cardunculus* plant extract and myricitrin were mixed with the compounds as mentioned in the above Table.

Seeds of *Arabidopsis thaliana* were sown in pots and the obtained plants were watered daily. When the plants had two leaf stages, these were sprayed with pelargonic acid (positive control used as commercial bioherbicide at 31 g/L), with distilled water (negative control), with the compounds of the formulation only (see Table 2) or with one of the compositions (a) and (e) according to the invention and described above. There were five treatments and three replicates per treatment maintained in a completely randomised manner. Four days after spraying, the treated weed plants were examined for visible injury levels in terms of wilting, necrosis and chlorosis. A percentage of efficacy was determined as followed: (number of wilted or necrotic leaves / total number of leaves) * 100.

The obtained results are presented in Figure 4. As it can be seen, all the compositions according to the invention present a post-emergence herbicidal activity. From the results shown in Figure 4, formulated *Cynara cardunculus* plant extract showed a high herbicidal effect, like the herbicidal

effect of pelargonic acid at 31 g/L. At 60 mg/L, the formulated myricitrin also presents a phytotoxic effect on *Arabidopsis thaliana*.

Statistical analysis

All statistical analyses were performed with Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA). Results were examined statistically using one-way analysis of variance (ANOVA) followed by Tukey multiple range tests, with different concentration of methanolic plant extracts. All determinations were conducted in triplicate. The differences between individual means were considered significant if $p < 0.05$.

The present invention has been described in terms of specific embodiments, which are illustrative of the invention and not to be construed as limiting. More generally, it will be appreciated by persons skilled in the art that the present invention is not limited by what has been particularly shown and/or described hereinabove.

Claims

Herbicidal composition comprising at least one phenolic active compound, characterized in that said at least one phenolic active compound is myricitrin.

Herbicidal composition according to claim 1, characterized in that said myricitrin is: from synthetic origin; or from an extract or from a fraction of an extract from the roots, the leaves, the stems, the fruits, the seeds and/or the flowers of at least one plant chosen from the group consisting of *Limoniastrum guyonianum*, *Peganum harmala*, *Ruta chalepensis*, *Ricinus communis*, *Nitraria retusa*, *Cynara cardunculus*, *Artemisia herba-alba*, *Mesembryanthemum edule*, *Tamarix gallica*, *Datura stramonium*, *Myrica esculenta*, *Myrica cerifera*, *Nymphaea lotus*, *Nymphaea odorata*, *Chrysobalanus icaco*, *Polygonum aviculare* and mixtures there of.

Herbicidal composition according to claims 1 or 2, characterized in that myricitrin is present in the composition at a concentration ranging from 0.1 mg/L to 10000 mg/L, preferably ranging from 0.25 mg/L to 5000 mg/L, more preferably ranging from 0.5 mg/L to 2500 mg/L, even more preferably ranging from 0.75 mg/L to 1500 mg/L, even more preferably ranging from 0.8 mg/L to 500 mg/L, even more preferably ranging from 1 mg/L to 100 mg/L.

Herbicidal composition according to any of preceding claims, characterized in that it further comprises at least one additional active compound chosen from the group consisting of syringic acid, p-coumaric acid, quercetin, naringenin, catechin, apigenin, gallic acid, cynarin acid, chlorogenic acid, cynaroside, luteolin, aguerin B, grosheimin, cynaropicrin and mixtures.

Herbicidal composition according to claim 4, characterized in that said at least one additional active compound is: from synthetic origin; or from an extract or from a fraction of an extract from the roots, the leaves, the stems, the fruits, the seeds and/or the flowers of at least one plant chosen from the group consisting of *Limoniastrum guyonianum*, *Peganum harmala*, *Ruta chalepensis*, *Ricinus communis*, *Nitraria retusa*, *Cynara cardunculus*, *Artemisia herba-alba*, *Mesembryanthemum edule*, *Tamarix gallica*, *Datura stramonium*, *Myrica esculenta*, *Myrica cerifera*, *Nymphaea lotus*, *Nymphaea odorata*, *Chrysobalanus icaco*, *Polygonum aviculare* and mixtures there of.

Herbicidal composition according to claims 4 or 5, characterized in that said at least one additional active compound is present in the composition at a concentration ranging from 0.1 mg/L to 10000 mg/L, preferably ranging from 0.2 mg/L to 5000 mg/L, more preferably ranging from 0.25 mg/L to 500 mg/L.

Herbicidal composition according to any of preceding claims, characterized in that the composition further comprises at least one solvent chosen from the group consisting of at least one natural lipophilic solvent, at least one synthetic lipophilic solvent, at least one natural hydrophilic solvent, at least one synthetic hydrophilic solvent, at least one natural amphiphilic solvent, at least one synthetic amphiphilic solvent and mixtures thereof.

Herbicidal composition according to claim 7, characterized in that said at least lipophilic solvent is chosen from the group consisting of animal oil, vegetable oil, petrochemical oil and mixtures thereof.

Herbicidal composition according to claim 8, characterized in that said vegetable oil is chosen from the group consisting of hazelnut oil, rapeseed oil, pine oil, soybean oil, sunflower oil, pistachio oil, olive oil, almond oil, sweet almond oil, peanut oil and mixtures thereof.

Herbicidal composition according to claim 7, characterized in that said at least hydrophilic solvent is chosen from the group consisting of water, alcohols, ketones, esters, ethers, polyols, ethyl acetate, propylene carbonate, acetonitrile and mixtures thereof.

Herbicidal composition according to claim 7, characterized in that said at least one natural amphiphilic solvent and/or said at least one synthetic amphiphilic solvent is at least one non-ionic surfactant.

Herbicidal composition according to 11, characterized in that said at least one non-ionic surfactant is chosen from the group consisting of sorbitan monododecanoate poly(oxy-1,2-ethanediyl), alkyl polyglucosides, sorbitan laurate, polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (20) sorbitan monopalmitate, polyoxyethylene (20) sorbitan monostearate, polyoxyethylene (20) sorbitan monooleate, polyoxyethylene glycol sorbitan laurate, hexaethylene glycol sorbitan monooleate, polyoxyethylene sorbitan stearate, decaglyceryl monooleate, decaglyceryl dioleate, polyoxyethylene

sorbitan tristearate, monodehydrosorbitol monooleate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan laurate and mixtures there of.

Herbicidal composition according to claims 11 or 12, characterized in that said at least one non-ionic surfactant presents a hydrophilic-lipophilic balance (HLB) value comprised between 5 and 7, preferably a HLB value equal to 6.

Herbicidal composition according to any claims 11 to 13, characterized in that said at least one non-ionic surfactant is present in the composition at a concentration ranging from 0.1 g/L to 500 g/L, preferably ranging from 0.25 g/L to 250 g/L, more preferably ranging from 0.5 g/L to 100 g/L, even more preferably ranging from 1 g/L to 50 g/L, even more preferably ranging from 2 g/L to 25 g/L.

Herbicidal composition according to any of preceding claims, characterized in that it further comprises at least one adjuvant chosen from the group consisting of paraffin wax, Trend® 90, Heliosol®, 1-octyl-2-pyrrolidone, Actirob® B, Atplus® 463, Actilandes™, Vegelix®, Emulsol® and mixtures there of.

Herbicidal composition according to any of preceding claims, characterized in that the composition is under the form of granules, liquids, emulsions or concentrates.

Use of a composition according to any claims 1 to 16 for obtaining phytotoxic activity comprising inhibiting seed germination, or inhibiting the development and growth of the roots, the development and growth of the hypocotyls, the development and growth of the epicotyls, the development and growth of the plantlets or the development and growth of aerial parts of the plant.

Abstract

Herbicidal composition comprising at least one phenolic active compound

The present invention relates to a herbicidal composition comprising at least one phenolic active compound being myricitrin. The invention also relates to the use of such a herbicidal composition.

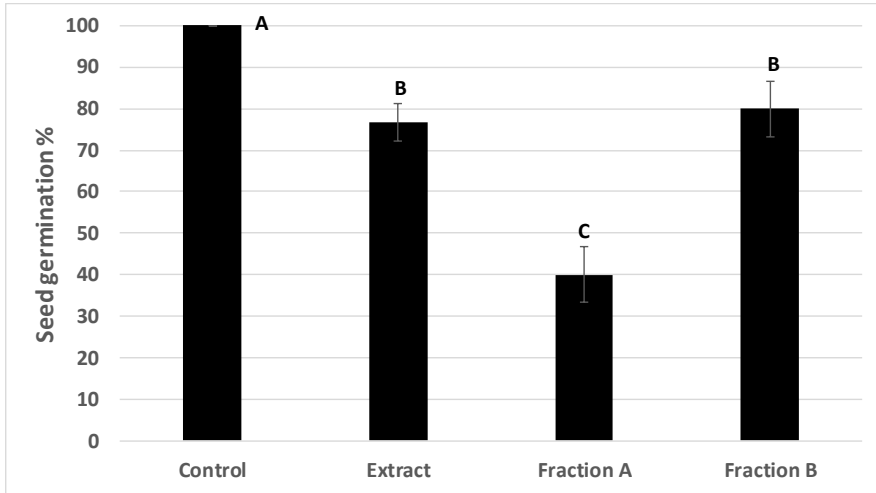


Figure 33 : shows the percentage of germination (seed germination %) after 5 days of the weed *T. incarnatum* treated by a *C. cardunculus* plant extract (Extract), a first fraction obtained from the *C. cardunculus* plant extract (Fraction A) and by a second fraction obtained from the *Cynara cardunculus* plant extract (Fraction B)

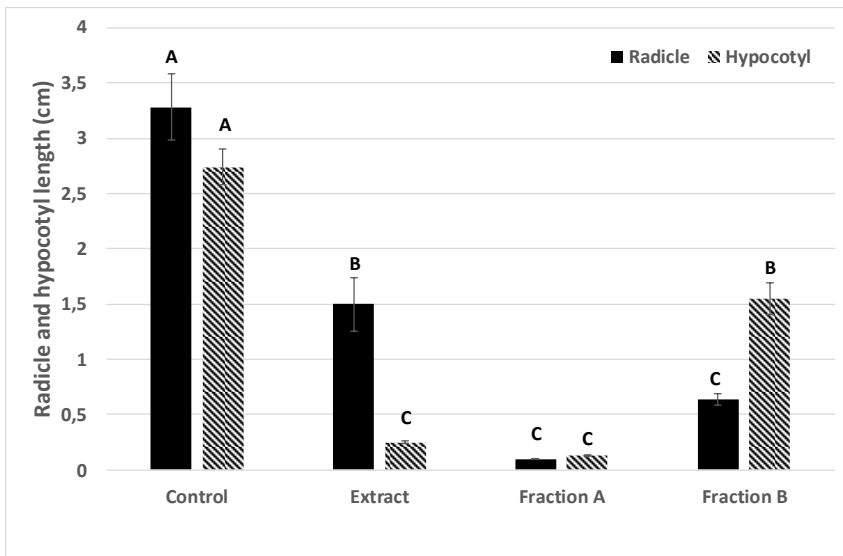


Figure 34 : shows the radicle and hypocotyl length (cm) after 5 days of the weed *Trifolium incarnatum* treated by a *C. cardunculus* plant extract (Extract), a first fraction obtained from the *C. cardunculus* plant extract (Fraction A) and by a second fraction obtained from the *C. cardunculus* plant extract (Fraction B)

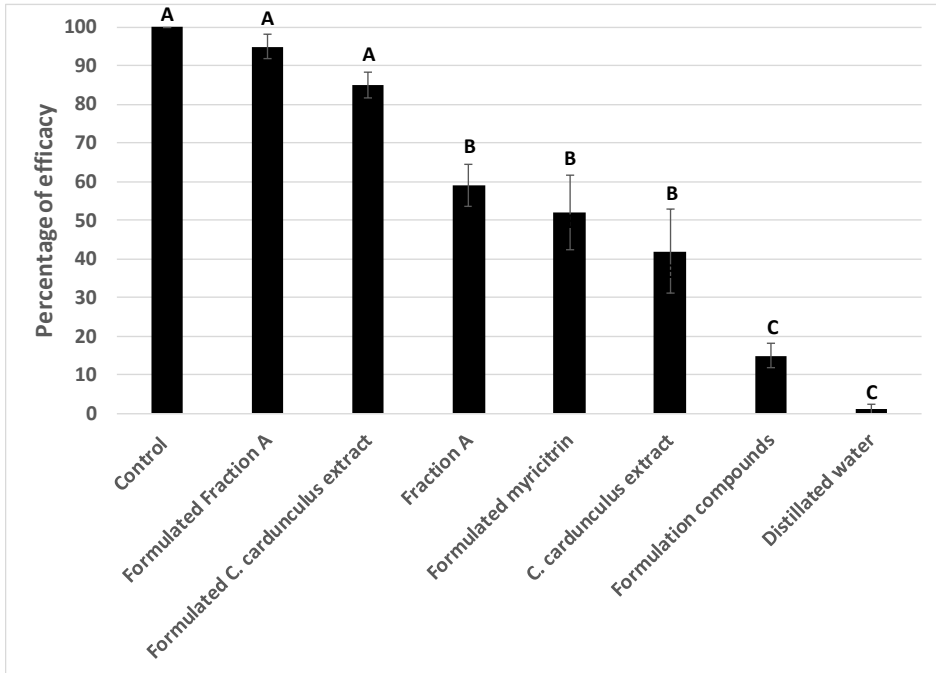


Figure 35 : shows the post-emergence activity (percentage of efficacy) of different compositions according to the invention on the treated weed *T. incarnatum* after 5 days: formulated *C. cardunculus* plant extract, *C. cardunculus* plant extract, formulated Fraction A of *C. cardunculus* plant extract, Fraction A of *Cynara cardunculus* plant extract and formulated myricitrin;

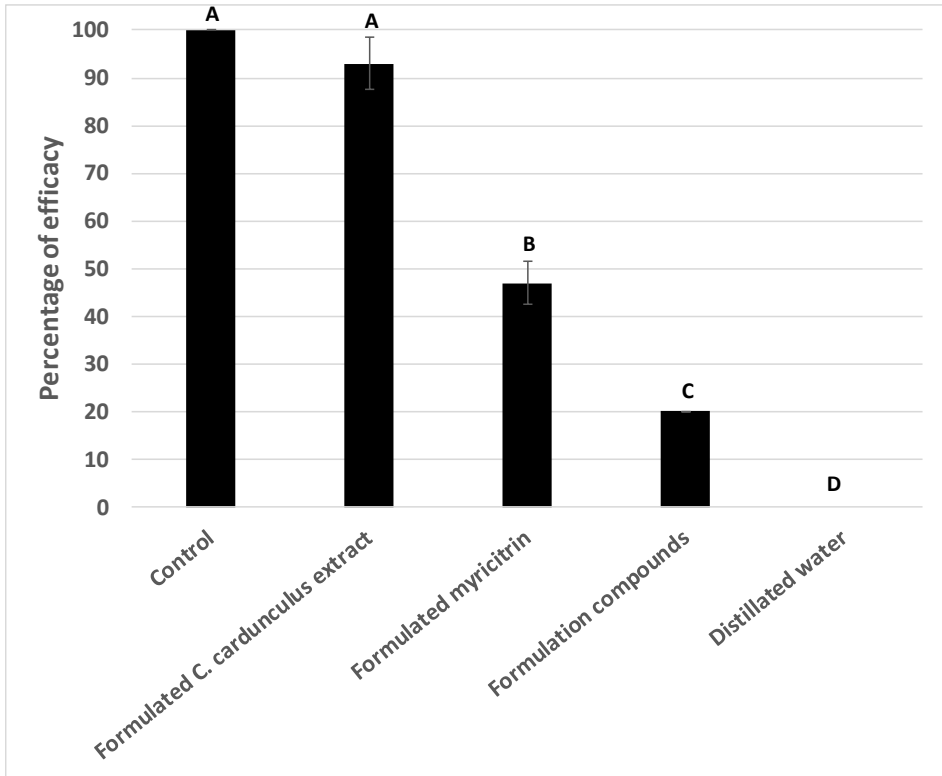


Figure 36 : shows the post-emergence activity (percentage of efficacy) of different compositions according to the invention on the treated weed *A. thaliana* after 4 days: formulated *C. cardunculus* plant extract and formulated myricitrin.

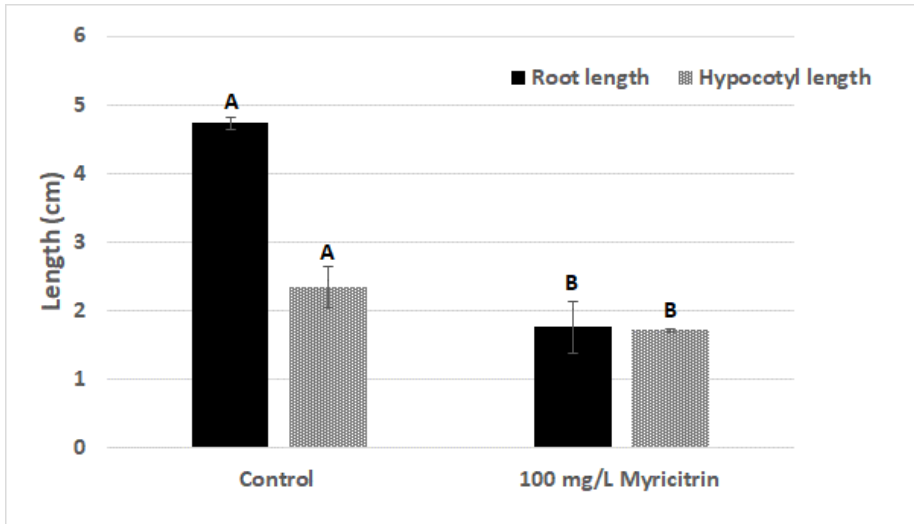


Figure 37: shows the radicle and hypocotyl length (cm) after 7 days of the weed *T. incarnatum* treated by a myricitrin solution at 100 mg/L in methanol 1%.

6

Discussion générale, conclusions et perspectives

1 Discussion générale et conclusions

Les plantes aromatiques méditerranéennes sont un bon exemple de l'utilisation des propriétés pesticides des plantes comme agents de lutte contre divers phytopathogènes. Au cours de cette Thèse de Doctorat, nous avons mis en évidence l'activité herbicide de plusieurs plantes originaires de la Tunisie. Cette flore Tunisienne, qui malgré sa grande richesse en espèces médicinales et aromatiques, reste très peu explorée et valorisée à ce jour. Ainsi, l'investigation phytochimique de cet héritage est d'une importance capitale non seulement pour la valorisation et la rationalisation de ces espèces, mais également pour la recherche des molécules potentiellement actives. Dans notre étude, il s'est avéré que certains extraits végétaux, et en particulier de ceux de *C. Cardunculus* et *R. officinalis*, contiendraient des composés chimiques inhibiteurs qui induisent des conditions de stress affectant la croissance générale des plantes

D'autre part, la Tunisie, située au niveau de plusieurs étages bioclimatiques (du subhumide au saharien), présente une large variabilité pédoclimatique. Elle abrite un très grand nombre de plantes médicinales et aromatiques dont plusieurs espèces sont extrêmophiles, telles que celles qui se trouvent dans des biotopes salins et arides (*Cakile maritima*, *Suaeda fruticosa*, *Tamarix africana*, etc...). Ces plantes spontanées sont souvent soumises aux contraintes permanentes de l'environnement, ce qui favorise l'accumulation d'une quantité importante de métabolites secondaires (polyphénols, huiles essentielles, etc.), pour surmonter les conditions extrêmement défavorables. D'autres part, très peu d'études ont montré que ces extraits végétaux pouvaient avoir un potentiel herbicide important (Kashkooli & Saharkhiz, 2014; Bouabidi et al., 2015 ; Lin et al., 2004 ; Yan et al., 2014 Poonpaiboonpipat et al., 2013 ;Kaur et al., 2012; Ben Ghnaya et al., 2013). Dans cette optique, les recherches menées dans le cadre de cette thèse de doctorat ont permis de sélectionner l'extrait phénolique de *Cynara cardunculus* et l'huile essentielle de *Rosmarinus officinalis*. Il est nécessaire de préciser aussi que la composition chimique de ces deux extraits a été étudiée. Cette composition peut varier en fonction de l'année de culture, du cultivar, de la localisation de la culture de la plante, de la méthode d'obtention de l'huile, du type de sol et de la période de récolte (Falleh et al., 2012 ; Kaloustian & Hadji-Minaglou,

2012,). Dû au grand nombre de constituants différents, ces extraits n'agissent pas uniquement sur un seul site d'action. Cependant, seulement deux publications récentes ont mis en évidence le pouvoir anti germinatif de *C. cardunculus* grâce à la présence des composés sesquiterpéniques comme l'aguérine B, la grosheimin et la cynaropicrine (Rial et al., 2014 ; Rial et al., 2016) . Dans notre étude, nous avons mis en évidence que des composés phénoliques sont à l'origine de l'activité herbicide de cet extrait. D'autre part, les huiles essentielles ont été très étudiées pour leurs pouvoirs herbicides mais il s'agit seulement de l'étude de leur efficacité sur la croissance des adventices dans les conditions du laboratoire.

Jusqu'à aujourd'hui, aucune formulation n'a été développée sur nos extraits végétaux afin d'améliorer l'efficacité de ceux-ci. De plus, les modes d'action végétaux et en plus les modes d'action qui permettraient l'élaboration d'une telle formulation en vue de les stimuler sont méconnus. Pour cette raison, cette étude pourrait représenter un travail original et introductif à une nouvelle thématique de recherche.

Le premier axe visait à déterminer le mode d'action des composés bioactifs des extraits végétaux identifiés. Nous avons bien montré que pour le cas de l'extrait de *C. cardunculus*, les composés phénoliques, plus particulièrement les flavonoïdes, pourraient interagir seulement avec la partie polaire de la membrane lipidique d'*A. thaliana*. La question de l'origine de la phytotoxicité peut notamment se poser. Est-ce que cette interaction superficielle pourrait être l'origine du stress. Dans notre étude, il s'est avéré que ces composés allélochimiques induisaient un stress oxydatif à l'intérieur des plantes cibles, provoquant une altération de tous les processus physiologiques et biochimiques de la plante. Dans ce contexte, Poonpaiboonpipat *et al.* (2013) ont montré que les huiles essentielles peuvent perturber la perméabilité des membranes cellulaires des adventices à cause de la pénétration de monoterpènes à travers la paroi et les membranes cellulaires. Cette perturbation cause une fuite de potassium qui inhiberait la respiration dépendante du glucose. D'autre part, l'augmentation de la perméabilité des membranes provoque aussi une augmentation de la fuite de solutés affectant par la suite les fonctions membranaires (Movileanu *et al.*, 2000). D'ailleurs une peroxydation lipidique et une fuite élevée d'électrolytes résultant en une perte d'intégrité membranaire sont parmi les facteurs clés qui déterminent les blessures cellulaires (Singh *et al.*, 2006). En effet, la membrane constitue l'une

des principales clés pour la détermination du mode d'action des composés bioactifs.

Après avoir identifié les molécules bioactives via un fractionnement bio-guidé, nous avons eu recours à l'étude de l'interaction des composés phénoliques identifiés dans *C. cardunculus* avec la membrane cellulaire en utilisant des outils de bioinformatique et biophysique. Il s'est avéré que ces composés n'interagissent qu'avec la partie polaire de la membrane. La naringinine, l'une de ces molécules bioactives, pénétrerait même dans la membrane et se « déploierait » latéralement par rapport à la surface, dans la partie des têtes hydrophiles de la bicouche lipidique. En effet, Erlejman et al. (2004) ont montré que ce type de molécule pourrait s'insérer dans des membranes biologiques de manière plus ou moins profonde en fonction de leur polarité. La naringinine a donc probablement un effet de perturbation locale dans la région polaire de la bicouche lipidique. Ceci a été aussi confirmé par Hendrich.(2006).

La myricitrine, un autre composé phénolique issu de *C. cardunculus* pourrait aussi présenter un léger effet de perturbation par son insertion superficielle dans la membrane modèle. L'insertion de la myricitrine et de la naringinine dans la membrane plasmique pourrait modifier la fluidité de celle-ci (Hendrich, 2006) et augmenter la sensibilité des lipides membranaires à une peroxydation lipidique. D'ailleurs, nous avons bien confirmé à travers notre étude que la naringinine augmente l'effet herbicide de la myricitrine par pulvérisation sur *T. incarnatum*. Schnitzer et al. (2007) confirment que des molécules antioxydantes peuvent agir comme inhibiteur ou promoteur de la peroxydation des lipides membranaires et être une cause de l'oxydation et du positionnement de l'antioxydant dans la membrane. Par rapport à l'interaction des huiles essentielles avec la membrane, Lins et al. (2019) ont montré que les monoterpènes phytotoxiques pourraient traverser le maillage de la paroi cellulaire et interagir directement avec la partie apolaire des lipides membranaires grâce à leurs longues chaînes d'hydrocarbures. Plusieurs auteurs supposent que les huiles essentielles (HE) agissent en provoquant des changements biochimiques et physiologiques dans la croissance des plantules (De Martino et al., 2010). Par exemple, les HE de citrates de *Cymbopogon* ont notamment ralenti l'activité de l' α -amylase dans les graines de *E. crus-galli* (Poonpaiboonpipat et al., 2013). De plus, l'huile essentielle d'*Artemisia* sp

induisait une production d'espèces réactives de l'oxygène, qui à son tour causait des dommages entraînant une peroxydation des lipides, une diminution de la fluidité de la membrane et finalement une augmentation des fuites membranaires et des récepteurs inactivés, des enzymes et des canaux ioniques (Kaur et al., 2012). Selon nos résultats préliminaires, l'utilisation d'HE dans la formulation des bioherbicides peut offrir de nouvelles perspectives pour la production durable et l'utilisation pratique d'HE. Certaines d'entre elles ont déjà été commercialisées et lancées avec succès dans l'agriculture biologique aux états-Unis. Ils perturbent la cuticule et contribuent à la dessiccation et la brûlure des tissus jeunes. Des exemples en sont le bioherbicide disponible dans le commerce portant le nom commercial de GreenMatch EX, constitué d'huiles de citronnelle (*Cymbopogon sp.*) Ou d'Interceptor™ contenant 10% d'huile de pin (*Pinus sylvestris L.*) (Soltys et al., 2013). En ce qui concerne l'Europe, aucune huile essentielle n'a été commercialisée en tant que bioherbicide. Il y'a seulement des HE commercialisées en tant que insecticides ou fongicides.

Dans les deux cas, le traitement de la plante cible que ce soit par un extrait phénolique ou une huile essentielle provoque un déséquilibre des processus physiologiques et biochimiques. Aucune étude n'a montré qu'une seule molécule dans ces extraits ou un seul site d'action dans la plante cible pourraient être à l'origine de ce déséquilibre (Ben kaab et al 2019). Effectivement, nos résultats ont montré que l'ajout de la naringénine peut augmenter l'effet herbicide de la myricitrine mais cet effet ne présente pas la totalité de l'activité de la fraction où ces molécules sont présentes. Ceci explique qu'il y'a sans doute d'autres molécules qui n'ont pas encore été identifiées dans l'extrait phénolique de *C. cardunculus* et qui participent à cette activité herbicide. Le mode d'action des extraits végétaux reste toujours difficile à déterminer au cours d'une seule étude mais en se référant à la littérature, nous avons créé un schéma récapitulatif hypothétique qui introduit la compréhension de ce mode d'action. En effet, plusieurs études ont confirmé que les flavonoïdes pourraient avoir un effet phytotoxique et être donc responsables de l'activité herbicide de l'extrait brut de *C. cardunculus*. Sakihama et al. (2002) ont montré que ces flavonoïdes pouvaient interagir avec les ROS dans les cellules et produire des composés pro-oxydants tels que des groupes phénoxy. Tout dépend de la plante cible. Ce dernier pourrait être détoxifié par des réactions enzymatiques et non enzymatiques. Dans certaines

conditions (changement de pH, présence de Al, Zn, Mg, Cu, Fe, Cd ou Ca), ces radicaux phénoxyles peuvent rester dans la cellule et être responsables de la peroxydation lipidique qui réduit la synthèse de protéines et d'ADN et aboutit par conséquent à la mort cellulaire (Eghbaliferiz et Iranshahi 2016; Decker, 1997; Sakihama et al. 2002). Dans notre étude, nous avons également examiné l'effet des principaux composés de notre extrait brut, seuls ou en combinaison. Nous avons ainsi montré que la myricitrine, la naringénine et la quercétine testées seules étaient des composés bioactifs (Ben Kaab et al., 2019). En plus de ces résultats, nous avons déterminé l'effet combiné de ces flavonoïdes et leur interaction avec la membrane lipidique de *A.thaliana*. En fait, la naringénine a augmenté l'effet herbicide de la myricitrine. Aucune étude n'a été menée sur l'effet de mélange herbicide de composés phénoliques. Cependant, Feng et al. (2019) ont montré que deux alcaloïdes d'amine, la sarmentosine et la sarmentine, isolés de *P. sarmentosum* avaient un grand potentiel en tant qu'herbicide botanique. Dans le même esprit, les mélanges de xanthoxyline et de trois autres composés naturels (alcène, aldéhyde phénolique et acide gras insaturé) dans une solution aqueuse de surfactant Tween® 80 ont considérablement inhibé la germination et la croissance des racines de l'amarante de Chine à de faibles concentrations. Ils ont confirmé que l'effet synergique de ces composés pourrait être appliqué au développement d'herbicides potentiels sans aucune résistance aux mauvaises herbes.

Comprendre la corrélation entre le mode d'action du *C. cardunculus*, de l'extrait brut de cardunculus et du stress oxydatif semble être l'un des aspects difficiles. En fait, dans notre étude nous confirmons que l'insertion de myricitrine et de naringinine dans la membrane plasmique pourrait modifier la fluidité de la membrane et augmenter sa sensibilité à la peroxydation lipidique. Cela pourrait augmenter les fuites d'électrolyte et la production de niveaux élevés de radicaux phénoxyles. Ces derniers sont parmi les origines du stress oxydatif qui perturbe de nombreux processus biochimiques chez les plantes. (Ben kaab et al 2020).

Le deuxième axe de recherche mené dans cette étude est la formulation d'un bioherbicide qui reste peu exploré dans des études précédentes. Des adjuvants de formulation comme le tween 20 et le span 80 sont visés pour renforcer l'efficacité des extraits végétaux et les composés actifs. En effet, très peu de

recherches ont été menées à propos de la formulation des bioherbicides à partir d'extraits végétaux. Uddin et al. (2014), ont mis en évidence une formulation d'un bioherbicide (poudre) à base du sorgoleone extrait du *Sorghum bicolor*. Cette formulation présente un effet herbicide en pré-émergence et post-émergence. De même, une nouvelle publication a montré qu'une nanoémulsion basée sur l'huile essentielle de *Satureja hortensis* présente un effet herbicide important (Hazrati et al., 2017a). L'originalité de cette thèse réside sur le fait qu'aucune étude n'a encore été menée sur la formulation d'un bioherbicide à partir d'un extrait phénolique de *C. cardunculus* (1) ni de l'huile essentielle de *R. officinallis* (2). Des travaux ont été réalisés au laboratoire de Phytopathologie Intégrée et Urbaine pour optimiser une formulation herbicide efficace en se basant sur la stabilité, la finesses et l'activité herbicide de cette formulation.

Il est nécessaire de mettre l'accent sur le caractère innovant des recherches menées dans le cadre de cette thèse. En effet, le fractionnement et l'analyse des résultats par HPLC de l'extrait phénolique de plante de *C. cardunculus* ont montré la présence de 5 composés phénoliques. Parmi ces composés, la myricitrine et la naringénine ont présenté des propriétés herbicides à faibles concentrations. La littérature décrit de nombreux effets biologiques des composés phénoliques. Même si les études sont moins nombreuses, certains composés phénoliques et flavonoïdes sont connus par leurs propriétés herbicides (Ribeiro et al., 2015; Nebo et al., 2014; Javaid et al., 2010; Watanabe et al., 2014; Hiwa, 2017). Martino et al. (2012) et Bido et al. (2010) ont montré que la naringénine peut inhiber la germination et la croissance des adventices. Par contre, aucune publication n'a été réalisée sur l'activité herbicide de la myricitrine. Ceci a fait l'objet de la publication d'une Demande de Brevet Internationale. D'autre part, les recherches scientifiques visant l'étude du potentiel herbicide d'extraits végétaux sont très limitées jusqu'à présent. En effet, plusieurs études se sont intéressées seulement à la présence ou à l'absence d'un effet herbicide pour certains extraits végétaux. Aucune étude pertinente n'a été réalisée afin d'élaborer un bioherbicide à base d'extraits végétaux et étudier par la suite son spectre d'action vis à vis d'une série d'adventices problématiques. Ceci présente aussi un caractère innovant dans cette thèse. D'autre part, nos résultats permettent de mettre en évidence deux produits naturels ayant un potentiel bioherbicide, Le premier est l'extrait de *C. cardunculus* qui présente une activité herbicide importante en post-

émergence. Le deuxième est l'huile essentielle de *R. officinalis* qui inhibe la croissance des plantes à 100 % en pré-émergence. Ces deux produits pourraient être utiles dans un schéma de traitement en lutte intégrée et biologique.

D'autre part l'application d'extraits de plantes en tant que bioherbicide de prélevée ou de post-émergence pourraient entraîner une réduction des doses d'herbicide synthétique en raison de leur action synergique ou additive. Il s'est avéré que la majorité des extraits phytotoxiques révélés dans la littérature scientifique ne sont pas toujours suffisamment efficaces pour inhiber totalement la croissance ou la germination des mauvaises herbes surtout lorsqu'ils sont appliqués en pulvérisation, même lorsque les plantes présentent un potentiel allélopathique élevé en tant que paillis, système de culture intercalaire ou en rotation (Pavela et al, 2016). Ceci peut être le résultat d'un effet inhibiteur de l'activité d'un composé par un autre dans une solution aqueuse ou l'impossibilité de pénétration de l'extrait à travers la cuticule. Pour cette raison l'amélioration de l'efficacité de ces solutions aqueuse naturels est associée à l'extraction et l'isolement d'allélochimiques individuels e sa comparaison avec des herbicides synthétiques (soltys, 2013). Dans ce contexte, Javaid et al. (2010) ont isolé des flavonoïdes de mangue (*Mangifera indica L.*) et ont montré qu'ils provoquaient le jaunissement des plantules de parthénium (*Parthenium hysterophorus L.*). De plus, les flavonoïdes purifiés à partir d'un extrait de racine de *Stellera chamaejasme L.*, recueilli en Chine, ont constitué des phytotoxines potentiellement importantes contre *A. thaliana* (Yan et al., 2014). Le fractionnement d'extraits d'éthanol *Derris urucu* a permis l'identification de trois nouveaux dihydroflavonols nommés urucuol A, urucuol B et isotirumaline, qui ont montré une activité herbicide potentielle (Da Silva et al., 2013).

L'élaboration d'une formulation d'un bioherbicide à base d'huile essentielle reste un défi original dans ce projet de recherche. Il est primordial de signaler que l'encapsulation est une bonne solution aux problèmes de dégradation et de perte d'activité des composés bioactifs contenus dans notre HE. L'enrobage des objets organiques protège les composés bioactifs des contraintes mécaniques et des facteurs environnementaux, tels que la lumière, l'oxygène, l'humidité, la température et d'autres composés réactifs (Răileanu et al., 2013). Cela permet une conservation plus efficace des HE et de leurs propriétés lors

de la manipulation, du traitement et du stockage. L'encapsulation permettra également une libération contrôlée en fonction du site et du temps des HE (Capelezzo et al., 2018). Un bon exemple a été étudié pour prolonger l'effet herbicide de HE de *M. pulegium*. En effet l'encapsulation des microporteurs de cellules de levure a été réalisée, ce qui a entraîné une protection thermique accrue de l'huile et un effet insecticide prolongé de 72 heures, par rapport à l'huile non encapsulée. La libération d'HE s'est produit par diffusion passive avec environ 65 heures pour libérer 81% de l'huile encapsulée (Kavetsou et al., 2019).

Selon Pavela et al, 2016, la commercialisation des bioherbicides à base d'extraits végétaux y compris les huiles essentielles parait fortement compliquée et cela est dû à la législation stricte, la faible persistance des effets et le manque de qualité et de quantités suffisantes de matériels végétaux à des prix abordables (Pavela et al , 2016).

Les HE sont produites dans 17 500 espèces aromatiques de plantes supérieures appartenant principalement à quelques familles, notamment les Apiacées, les Myrtacées, les Lauracées, les Lamiacées et les Astéracées. Cependant, seule une faible proportion (environ 300 espèces) a trouvé une utilisation dans des applications commerciales (renaults et al 2015). De nombreuses HE prometteuses proviennent de plantes dont la culture est coûteuse ou désavantageuse en raison des faibles rendements en HE. Même les plantes qui sont actuellement cultivées pour la production commerciale d'HE ne peuvent pas toujours être cultivées facilement. L'une des raisons est que le processus physiologique du métabolisme secondaire des plantes peut varier entre les stades de développement. De plus, les proportions de mono- terpènes dépendent de la température et varient selon la phase phénologique de la plante (Benneli et al 2016). Enfin, l'acidité et le climat du sol (chaleur et humidité) affectent directement le métabolisme secondaire et la composition en HE. Pour relever ce défi, des produits d'élicitation, des manipulations génétiques et de nouvelles technologies pour la culture des plantes ont été suggérés, visant à augmenter la production et à normaliser les paramètres qualitatifs et quantitatifs des HE (Pavela et al, 2016). De nouvelles méthodes pour isoler les HE des plantes ont également été étudiées. À l'heure actuelle, les HE sont isolées des plantes en utilisant des méthodes classiques (c'est-à-dire par distillation standard du matériel végétal). L'investissement dans les nouvelles technologies (par exemple, les ultrasons, les micro-ondes) au cours

des dernières décennies a conduit à l'émergence de procédés d'extraction innovants et plus efficaces. D'après Singh 2014, les extraits végétaux sont confrontés à plusieurs opposants parmi lesquels, les membres de la Commission européenne responsables des processus d'autorisation, qui considèrent que certains sont plus dangereux que les composés synthétiques. Ces opposants utilisent le manque de données toxicologiques pertinentes pour les produits naturels comme argument contre leur utilisation. (Bakkali et al., 2008). Ces nouvelles tendances dans la recherche sur les plantes aromatiques, ainsi que le choix de chimio-types appropriés avec des rendements élevés ou une meilleure efficacité biologique ouvriront de nouvelles perspectives pour la production agricole durable et l'emploi pratique des extraits végétaux.

D'autre part, la toxicité des HE est généralement due à la présence de composés phénoliques, d'alcools et d'aldéhydes qui endommagent la membrane plasmique ou la membrane des organites et agissent comme pro-oxydant sur les protéines et l'ADN (Sacchetti et al., 2005). Les HE peuvent agir directement sur les mitochondries et perturber les membranes mitochondriales, ce qui modifie l'activité de celle-ci qui produisent alors des radicaux libres endommageant les lipides, les protéines et l'ADN (Bakkali et al., 2008).

Les processus d'autorisations des bioherbicides sont complexes et coûteux, tout comme l'autorisation de tout composé nouvellement synthétisé sans antécédents d'utilisation dans l'industrie alimentaire, cosmétique ou pharmaceutique. Les autorisations dans les États de l'UE nécessitent une documentation de sécurité par le biais d'études toxicologiques appropriées. Cependant, dans de nombreux cas, de telles études n'existent pas et leur préparation est trop coûteuse pour les fabricants locaux. La fabrication de bioherbicide, souvent d'importance locale seulement, est généralement à petite échelle car la production est limitée par la disponibilité de substances actives. (Pavela et al 2016).

2 Perspectives de la thèse

Plusieurs perspectives pourraient être intéressantes afin de mieux valoriser le travail réalisé au cours de cette Thèse.

Tout d'abord, la formulation constitue un volet important pour la continuité de cette thèse. Elle pourrait être envisagée sur un cocktail de molécules bioactives identifiées dans nos extraits végétaux. En plus la sélection d'autres adjuvants, d'une part, pourrait stabiliser mieux la formulation et augmenter l'efficacité et pourrait, d'autre part, protéger la substance active des radiations UV et diminuer la volatilité dans le cas des HE. Finalement, le choix d'adjuvants uniquement biosourcés donne une cohérence totale au bioherbicide. Dans un deuxième temps, ces formulations stables seront testées au champ ainsi que les études portant sur la toxicité et l'éco-toxicité de ces formulations seront menées afin de déterminer les risques potentiels de ces formulations sur la santé humaine, animale et sur l'environnement. Une méthode de mesure d'efficacité sera développée via le logiciel Matlab afin de mesurer le pourcentage des feuilles flétries/nécrosées par rapport aux pourcentages des feuilles totaux.

Dans cette thèse de doctorat, nous avons pu identifier les composés phénoliques principaux de l'extrait phénolique de *Cynara cardunculus* qui étaient actifs. L'efficacité de la myricitrine était à 40 % qui ne représente pas l'effet herbicide de la fraction active. Ceci s'explique sans doute par le fait qu'il y'aurait d'autres composés actifs non encore identifiés. Dans ce cas, il sera fortement recommandé d'identifier et purifier d'autres molécules et les tester sur des adventices en pré et post-émergence.

D'autre part, la détermination du mode d'action complet est une clé principale pour le développement d'un bioherbicide. En effet, il serait nécessaire de déterminer le potentiel de traversée des différents flavonoïdes à travers la cuticule. Aucune information n'a été trouvée à propos du pouvoir de pénétration des flavonoïdes à travers cette structure dans la littérature. L'identification du ou des modes d'actions précis associé(s) à chaque flavonoïde pourrait / pourraient être envisagé(s) dans le but de mieux comprendre leur activité biologique au niveau des cellules végétales. A ce titre, plusieurs pistes de modes d'actions potentiels sont rappelées ci-dessous : Potentiel de traversée de la membrane plasmique via des transporteurs membranaires ; Effet direct sur l'ADN ; Impact sur la mitochondrie

(respiration et membrane) ; Action sur la membrane thylakoïde ; Perturbation de l'activité des protéines membranaires ; Production de radicaux libres dans la cellule ou dans l'environnement de celle-ci qui engendrerait un stress oxydatif généralisé avec peroxydation lipidique, dégradation des protéines et de l'ADN.

Les feuilles de la plante cible traitées par les différents produits prometteurs pourraient être analysées par microscope électronique (transmission electron microscopy). Différents organites ainsi que les différentes membranes et parois seront ciblés comme : la cuticule, la paroi cellulaire, la membrane plasmique, le chloroplaste, la mitochondrie, le noyau, l'appareil de golgi, et le réticulum endoplasmique

Productions scientifiques

Sofiene Ben kaab Haissam Jijakli Riadh Ksouri Olivier Parisi, Simon Dal Mas. Herbicidal composition comprising at least one phenolic active compound. Brevet international WO2019162388 (A1) — 2019-08-29 .

Sofiene Ben kaab, Iness Bettaieb Rebey, Marwa Hanafi, Chadi Berhal, Marie laure Fauconnier, Caroline De Clerck, Riadh Ksouri, Haissam Jijakli (2019) Study of *Rosmarinus officinalis* essential oil as effective antifungal and herbicide agent. *Spanish journal of agriculture research* .

Sofiene Ben kaab, Iness Bettaieb Rebey, Marwa Hanafi, Khouala Mkhadmini, Abderrazak Smaoui Marie laure Fauconnier, Caroline De Clerck, Haissam Jijakli, Riadh Ksouri (2019) Screening of Tunisian plant extracts for herbicidal activity and formulation of bioherbicide based on *Cynara cardunculus* *South African Journal of Botany* .

Sofiene Ben kaab, Laurence Lins, Marwa Hanafi, Iness Bettaieb Rebey, Magali deleu, Marie laure Fauconnier, Caroline De Clerck, Haissam Jijakli, Riadh Ksouri Study of the phytotoxic effect of *Cynara cardunculus* extract and the interaction of its active molecules with the membrane. *Biomolecules* .

Iness Bettaieb Rebey Wissem Aidi Wanne **Sofiene Ben Kaab** Soumaya Bourgou Moufida Saidani Tounsi Riadh Ksouri Marie Laure Fauconnier (2019) Bioactive compounds and antioxidant activity of *Pimpinella anisum* L. accessions at different ripening stages *Scientia Horticulturae*

Amine kaddes, Olivier Parisi, Chadi Berhal **Sofiene ben kaab** Marie laure Fauconnier Bouzid Nasraoui , Haissam Jijakli, Sebastien Massart and Caroline De Clerck (2016) Evaluation of the effect of two volatile organic compounds on barley pathogens *Molecules*.

Iness Bettaieb Rebey, Soumaya Bourgou, **Sofiene Ben Kaab**, Wissem Aidi Wanne, Riadh Ksouri, Moufida Saidani Tounsi, Marie Laure Fauconnier (2019) On the effect of initial drying techniques on essential oil composition, phenolic compound and antioxidant properties of anise (*Pimpinella anisum* L.). seeds *Journal of Food Measurement and Characterization*.

Communications orales et posters

Sofiene ben kaab, Ines Bettaieb Rebey, Marie Laure Fauconnier, De clerck Caroline, Riadh ksouri and Haissam Jijakli (2018) Study of Tunisian plant extracts as bioherbicide. International symposium on Crop protection Gent.

Sofiene ben kaab, Riadh ksouri, Haissam Jijakli (2017) Essential oil of Tunisian plant: biopesticide applied in agricultural system. International Symposium on Crop Protection Gent.

Sofiene ben kaab, Olivier Parisi, De Clerck Caroline, Ksouri Riadh, and Haissam Jijakli (2016) Extremophile plants as source of biopesticides against European damageable plant pathogens. XIV Meeting of the IOBC-WPRS Working Group Biological Control of Fungal and Bacterial Plant Pathogens.

Sofiene ben kaab Olivier Parisi De Clerck Caroline Ksouri Riadh and Haissam Jijakli (2016) Herbicidal activity of Tunisian plant extracts against various weeds. International symposium on Crop protection Gent.