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Ministry of Higher Education and Scientific Research

جامعة الإخوة منتوري قسنطينة ا
Frères Mentouri Constantine I University
Université Frères Mentouri Constantine I

Faculty of Natural and Life Sciences
Animal Biology Department

كلية علوم الطبيعة والحياة
قسم بيولوجيا الحيوان

Master's degree thesis

Domain: Natural and Life Sciences
Field: Biological sciences
Specialty: Insects biology and population control

Order N°:
Serial N°:

Title:

Utilizing *Apis mellifera's* Venom as a Biological Control Agent against Vespid parasites (*Vespula germanica*)

Presented by:

BOULKROUNE Mohamed Habib Errahmene

On: 20/06/2023

Evaluation jury:

President of the jury: CHAIB Aouatef (MCB - UFMC 1).

Supervisor: BAKIRI Esma (MCB - UFMC 1).

Examiner: RAMLI Iman (MAA- UFMC 1).

Academic year

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Acknowledgement

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

First, I offer my heartfelt gratitude to Allah, the all-merciful, the source of all blessings and guidance. Alhamdulillah, I acknowledge your infinite wisdom and benevolence, which have illuminated my path and bestowed upon me the strength and determination to complete this thesis.

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Dedication

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

This thesis is not only a culmination of my academic journey, but it also represents a dream and a promise. By God's will, I stand one step away from achieving a goal that has consumed my thoughts and aspirations.

To my beloved late father **BOULKROUNE FAOUZI**, whose absence has been deeply felt throughout this journey, I dedicate this thesis in loving memory. Your guidance, wisdom, and unconditional love have shaped my character and fueled my ambitions. I sorely miss your presence, but I find solace in the knowledge that your spirit lives on within me. This work stands as a tribute to your lasting impact on my life.

To my loving **Mother**, my guardian angel, I dedicate this thesis with heartfelt appreciation. Your unwavering support, love, and guidance have been the pillars of my strength. You have selflessly nurtured my dreams, encouraged my pursuits, and believed in me even when I doubted myself. This dedication is a testament to your invaluable presence in my life.

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To my family and friends, whose unwavering love, encouragement, and understanding have sustained me throughout this journey, I dedicate this thesis. Your presence has been a constant source of motivation, and your belief in my abilities has bolstered my confidence. I extend my sincerest gratitude to all of you for your unwavering support.

Lastly, I dedicate this work to **myself**, reaffirming the dream that burns within me and promising to achieve it. I recognize the strength and resilience that resides in me, and I vow to utilize every ounce of my potential to overcome any obstacles that may arise on the final leg of my journey.

With gratitude, determination, and a renewed sense of purpose, I dedicate this thesis to the dream that awaits, vowing to do everything within my power, **by God's will, to achieve it.**

BOULKROUNE MOHAMED HABIB ERRAHMENE

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Introduction:

Parasitic vespid species, such as Vespula germanica, pose significant challenges due to their aggressive behavior and potential harm to humans, agricultural crops, and ecosystems (Smith et al., 2018; Johnson, 2020). Traditional methods of vespid control often rely on chemical pesticides, which can have detrimental effects on the environment and non-target organisms). In recent years, there has been growing interest in exploring alternative and environmentally friendly solutions for managing vespid populations . One such solution is the utilization of bee venom derived from Apis mellifera as a control agent. Bee venom is known to contain a diverse array of bioactive compounds, which exhibit potent antimicrobial, anti-inflammatory, and cytotoxic properties. This study aims to investigate the potential of bee venom as an effective and sustainable approach to control parasitic vespid species, specifically Vespula germanica.

The escalating concerns over the environmental and health impacts of chemical pesticides have led researchers and practitioners to seek alternative strategies for pest management. Integrated pest management (IPM) approaches, which prioritize environmentally friendly and sustainable practices, have gained momentum. Within the realm of IPM, the utilization of naturally occurring substances holds immense potential. Bee venom, obtained from Apis mellifera, has emerged as an intriguing candidate due to its biological properties.

The composition of bee venom comprises various bioactive components, including peptides, enzymes, and amines. These components contribute to its potent antimicrobial, anti-inflammatory, and cytotoxic effects (Smith and Johnson, 2020). However, the potential of bee venom as a control agent against parasitic vespid species remains relatively unexplored.

Against this backdrop, the investigation of bee venom as a control agent against Vespula germanica holds considerable significance. By harnessing the naturally occurring bioactive compounds in bee venom, Moreover, the eco-friendly nature of bee venom aligns with the principles of integrated pest management, promoting a balanced ecological system.

However, before bee venom can be adopted as a practical solution, it is crucial to address several key factors. Standardized production methods, dosage optimization, and effective application techniques need to be explored and refined. Additionally, comprehensive assessments of the safety and efficacy of bee venom in controlling parasitic vespid species are necessary.

This study aims to fill the existing research gap by examining the potential of bee venom derived from Apis mellifera as a control agent against Vespula germanica. By investigating the biological properties of bee venom, this research endeavors to contribute to the development of sustainable and environmentally friendly strategies for vespid control.

In this manuscript first, we present a review of the literature, enabling us to situate and place this group of insects in the world and Algeria. The second chapter is devoted to methodology. The third chapter is devoted to the results, and the final chapter to discussion and conclusion.

References:

CHAPTER I: LITERATURE REVIEW

CHAPTER I: LITERATURE REVIEW

I Generality on the sub-family of Vespinae

The Vespinae are a subfamily of social wasps in the family Vespidae. The group consists of four genera: Vespa (hornets, 22 known species), Provespa (nocturnal hornets, three species), and Dolichovespula and *Vespula* (yellowjackets, respectively 19 and 23 species). Phylogenetic relationships among the four genera have been the subject of argument, without any decisive conclusion.

These are medium-sized to gigantic insects, ranging from 10 to 40 mm in total body length. They have large kidney-shaped eyes and strong mandibles. The first gastral (second abdominal) segment is basally truncate with a vertical anterior face, unlike other social wasps in which it is gradually narrowed toward the base or petiolate. Both queens and workers have a fully developed.

Venom apparatus and an adeptness in stinging, making them at times of medical/veterinary concern.

The ground body color is light brown in the three Provespa species and black in most other species, but in the latter the body is marked with white, yellow or orange to varying extents. The typical yellow/orange-banded pattern on the gaster is believed to function as a warning signal that causes the wasps to be avoided by land vertebrates, especially birds. With this pattern many hornets and yellowjackets are models for other insects (e.g., some longhorn beetles, cessid moths, syrphid flies), which can thus avoid predation by birds (Batesian mimicry). Sympatric hornet and yellowjacket species often resemble each other in color pattern, thereby enhancing the efficiency of their defense against predators (Muellerian mimicry).

I-1 Distribution

The group as a whole occurs mainly in the Eurasian continent, throughout the islands of Southeast Asia to New Guinea, and throughout North America. With some natural extension into North Africa, some of the South Pacific islands and Central America.

Several species have also increased their ranges through human-facilitated introduction. As an example, the European hornet, *Vespa crabro*, was introduced into the USA in the mid-nineteenth century and is now widespread in eastern North America. Recently the yellow-legged hornet *Vespa velutina* has appeared in several countries in Western Europe and northeastern Asia Among species of *Vespula*, *Vl. germanica* and *Vl. vulgaris* have been introduced from Europe into North and South Americas, Australia, New Zealand, and (*Vl. germanica* only) South Africa. The Nearctic *Vl.*

pensylvanica is now present in nonnative areas in North America and is found in Hawaii. To date, no *Provespa* or *Dolichovespula* species appears to have been introduced outside its natural range.

I-2 Daily Activity

Most species are diurnal. As a rule, foraging usually starts one to two hours before sunrise and ends shortly after sunset, with the exception of *Vespa crabro* in which foraging continues during the night on a much reduced scale. However, the outside activity is also dependent on ambient tem- perature (2 °C as a minimum for yellowjackets), weather, and the availability of moonlight . Within the nest workers are awake at all hours. In contrast, *Vespa binghami* and all the species of *Provespa* are nocturnal and are characterized by conspicuously large ocelli, simple eyes that are sensitive to low light levels.

I-3 Food choice:

All vespines are predaceous on a wide range of insects and some other invertebrates, with variation among species. For this reason, some species can be regarded as beneficial for their roles in controlling forest and agricultural pests (Fig. 1).

Most Vespa species are predators of insects and other arthropods, but some are more or less specialized in particular prey, for example the immatures of paper wasps (Polistinae) in V. ducalis (Fig. 2). The giant hornet Vespa mandarinia and some other Vespa species often attack colonies of other social wasps, as well as hives of honeybees, causing serious damage to apiculture. Species of the Vespula rufa group and Dolichovespula hunt only live insects and

Other arthropods, but most Vespula species are scavengers on carrion as well as skillful hunters of live insects. (Fig. 3). Little information is available for Provespa; workers capture insects attracted to lights in residential areas.

All vespine wasps take carbohydrate food, such as nectar, tree sap, honeydew and fruit. Vespa mandarinia is able to monopolize sap-rich trees against other hornets, beetles and butterflies (Fig. 4). Workers solicit and take a lot of fluid regurgitated by larvae containing sugar and amino acids that are necessary for flight and other activities.

CHAPTER I: LITERATURE REVIEW

FIGURE 1: A *Vespa simillima* worker capturing a grasshopper. © Masao Nakamura

FIGURE 3: *Vespula shidai* foragers collecting meat from an abandoned dead fish.

FIGURE 2: A nest of a paper waspplundered by a female *Vespa ducalis*

FIGURE 4: Giant hornet *Vespa mandarinia workers occupying a sap-secreting site on a tree trunk. © Masao Nakamura*

I-4 Larval Development

Like other social wasps, vespines develop from eggs through larvae and pupae into adults. The number of ovarioles in each ovary ranges from 6 to 12 in queens of 23 studied species of hornets and yellowjackets. Workers may have the same or smaller numbers of ovarioles. Eggs are elongate, ovoid, milky white in color, and vary in size among species. In Vespula flaviceps, one of the smallest species, the major axis of mature oocytes measures around 2.2 mm long, while in *Vespa mandarinia*, the largest hornet, it is 5.7 mm long. Eggs are attached to the upper (basal) portion of cell walls by a short stalk. The larval stage con- sists of five instars . Larvae have no legs, and their eyes and antennae are vestigial even in the final-instar; they are just digestive sacs with a mouth (and its sensilla), salivary (labial)

glands, gut, Malpighian tubes, and respiratory sys- tem, other organs being of little importance at that stage of life. However, they change in their morphology, especially in mandibular shape, and behavior with growth. During the first- instar, when the larvae receive only liquid food, the mandibles are small and very fragile, but later the stronger mandibles are used for taking in solid food. The first to fourth-instar larvae are connected with the cell wall by casted skins, but after molting into the fifth instar they keep their position in the cell by their body volume that fills the cell. In hornets the final-instar larvae use the mandibles to communicate their hunger to workers by scraping cell walls to produce a sound even audible to human ears. Before pupating, the full-grown larva spins a white cocoon that covers part of the wall and the opening of each cell.

I-5 Systematic

According to Carpenter (1982) the family Vespidae is divided in six sub- families (Fig. 5) showing different lifestyle and biology. Euparagiinae and Masarinae include only solitary species. On the other hand, the Eume- ninae, the largest subfamily with more than one thousand species, presents a range of parental behaviors that is particularly important for the study of the origin of eusociality.

The Stenogastrinae wasps are eusocial but due to the low levels of social organization, they represent a key group for the evolution of social behavior (Hunt, 2007; Turillazzi, 1989, 1991). These wasps live in tropical forests of South East Asia and New Guinea where they build their nests without peduncles on trees, on rootlets hanging from earth trenches or on the walls of caves perfectly camouflaged with leaves, sticks, or mud pieces. Their colonies are very small with maximally a dozen individuals (Turillazzi, 1991). The subfamily comprises seven genera (Carpenter and Starr, 2000) with more than 70 described species (Turillazzi, unpublished).

The eusocial subfamily Polistinae is the most diversified group of social wasps, both in terms of number of species, morphological and behavioral diversity (Carpenter, 1991). Polistine social wasps are cosmopolitan, although concentrated primarily in the tropics, especially in the New World. They are divided in four tribes (Polistini, Myschocittarini, Ropalidiini, Epiponini), comprising 28 genera and more than 800 species (Carpenter, 1991) with a variable population size ranging from a few individuals to more than one million (in the colonies of the South American species Agelaia vicina). Accord- ing to the colony foundation strategy they are divided in: ‘‘independent- founding’’ species, when the nest is founded by solitary fertilized females (which can be joined by other fertilized females), and ‘‘swarm-founding’’ species, when a new nest is founded by a swarm composed by one

(or more) fertilized females, together with a group of workers or sterile females (Jeanne, 1991). Polistes is the only genus of the tribe Polistini and it comprises exclusively independent-founding species. This genus represents a key model for the study of social evolution because it shows only slight caste differentiation. Moreover, the wide geographical distribution, the limited size of their colonies and the nest without envelope suspended by a pedicel, make the genus Polistes a suitable model for scientific researches (Starks and Turillazzi, 2006; Turillazzi and West-Eberhard, 1996). All the species belong- ing to the genus Mischocyttarus, the only representative of the South American tribe Mischocyttarini, are independent-founding species (Gadagkar, 1991). The tribe Ropalidiini, instead, shows both modes of colony founding: the genus Parapolybia (Asian distribution) includes only independent-founding species as well as the African genus Belonogaster. The genus Polybioides has both African and Asian species all of which are swarm-founding. The genus Ropalidia is distributed both in Africa and in the South Asian and Australian tropics and includes both independent- and swarm-founding species (Gadagkar, 1991). The last tribe, the South American Epiponini, includes 24 swarm-founding genera (Jeanne, 1991).

The subfamily Vespinae (yellowjackets and hornets) presents only highly eusocial species and this implies morphological differentiation between queens and workers. They are mainly distributed in the temperate areas of the Old and New World where they build large nests with envelopes which protect a pile of cell combs where up to thousands individuals live. The subfamily includes four genera: Provespa, Vespa, Dolichovespula and Vespula (Greene, 1991; Matsuura, 1991; Spradbery, 1973).

Many morphological, developmental, and behavioral differences shown by Stenogatrinae wasps with respect to the other two eusocial subfamilies (resumed in Hunt, 2007; Turillazzi, 1991), together with biomolecular evidences (Hines et al., 2007; Schmitz and Moritz, 2000), may suggest that the similarities in the social biology among the three subfamilies can be due to evolutionary convergences. In this case, that the evolution of social behavior in these wasps could be an independent evolutionary pathway to eusociality in the family Vespidae (see Hunt, 2007; but see Pickett and Carpenter, 2010; Fig. 6).

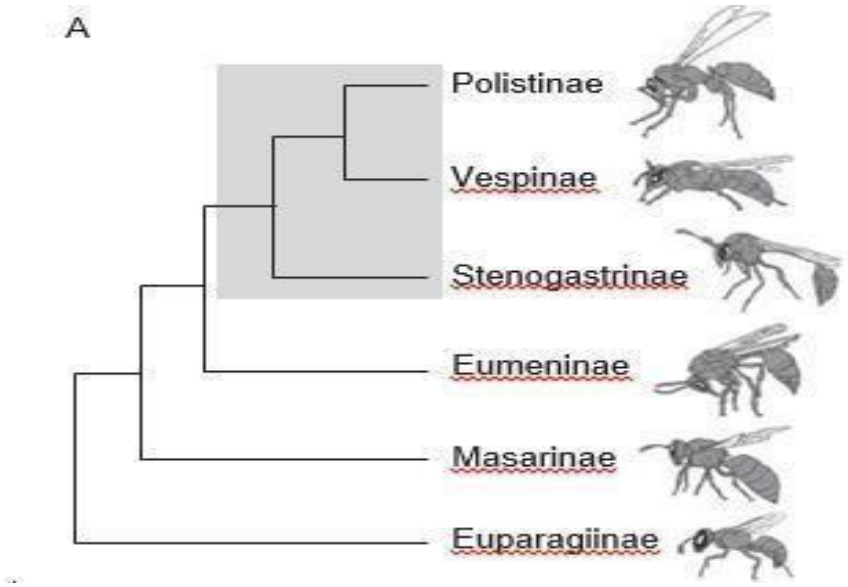

FIGURE 5: the phylogenetic tree based on morphological and behavioral characters (Carpenter, 1982; Pickett and Carpenter, 2010).

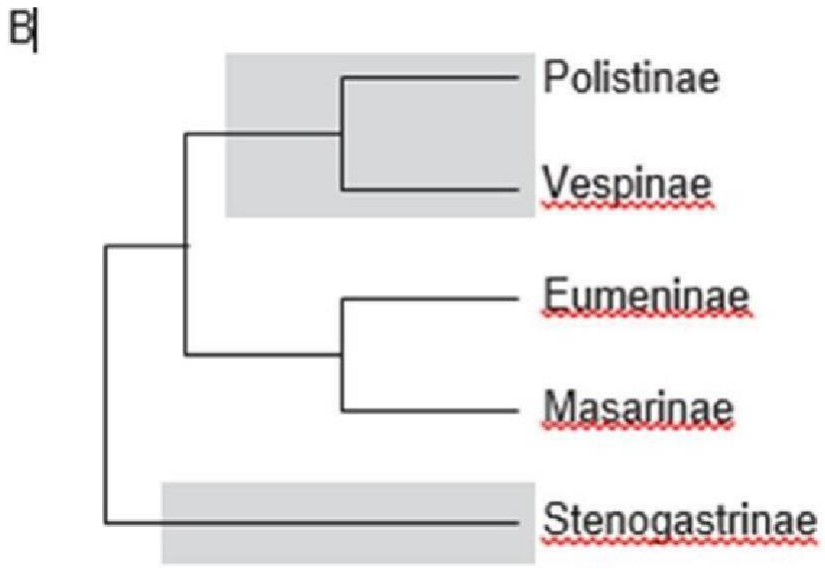

FIGURE 6: a more recent phylogenetic tree based only on molecular data (Schmitz and Moritz, 2000) (from Hines et al., 2007, Proc. Natl. Acad. Sci., Copyright (2007), National Academy of Sciences,

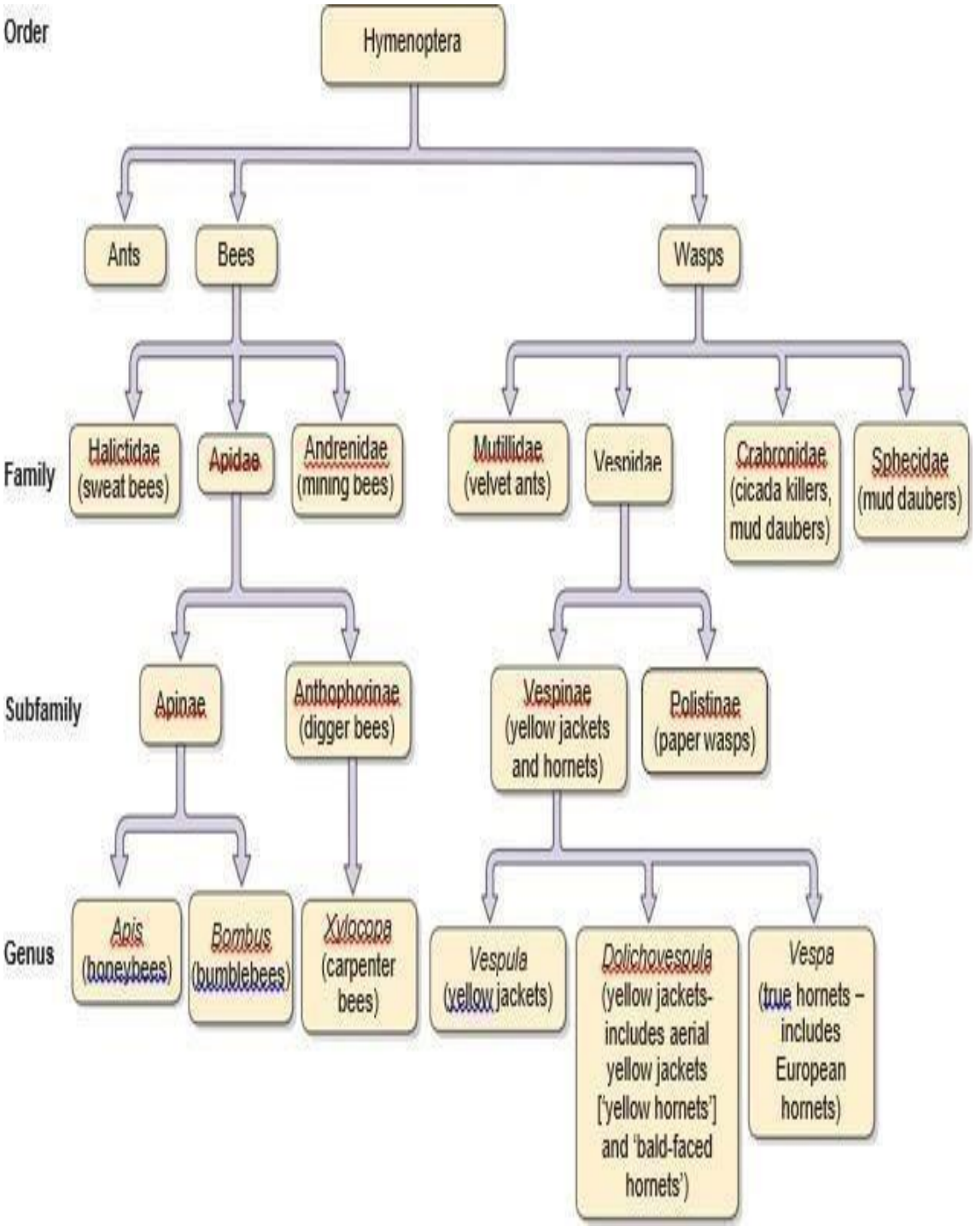

FIGURE 7: Overview of taxonomy of common Hymenoptera (stinging insects).

CHAPTER I: LITERATURE REVIEW

II Generality on *Vespula germanica*

II-1 Classification

Vespula germanica , or the European wasp, is a member of the Vespidae, a family that comprises both highly eusocial and solitary nesting wasps. Most species in the subfamilies Euparigiinae, Masarinae and Eumeninae are either solitary or presocial (see Table 1 for definition) (Cowan 1991), the Sterogastrinae are subsocial and parasocial (Turillazzi 1991), the Polistinaeprimitively social (Gadagkar 1991; Jeanne 1991; Reeve 1991), while the Vespinae are highly eusocial (Spradbery 1973; Edwards 1980). The genus Vespa comprises the hornets, while Dolichovespula and Vespula are commonly referred to as 'yellow jackets' (mostly in the U.S.A.). *Vespula germanica* belongs to *the V. vulgaris* group, which, apart from morphological differences, is often considered to be the 'scavenger group' within the genus (Akre et al. 1981; Carpenter 1987). (See Figure 7 for relationships)

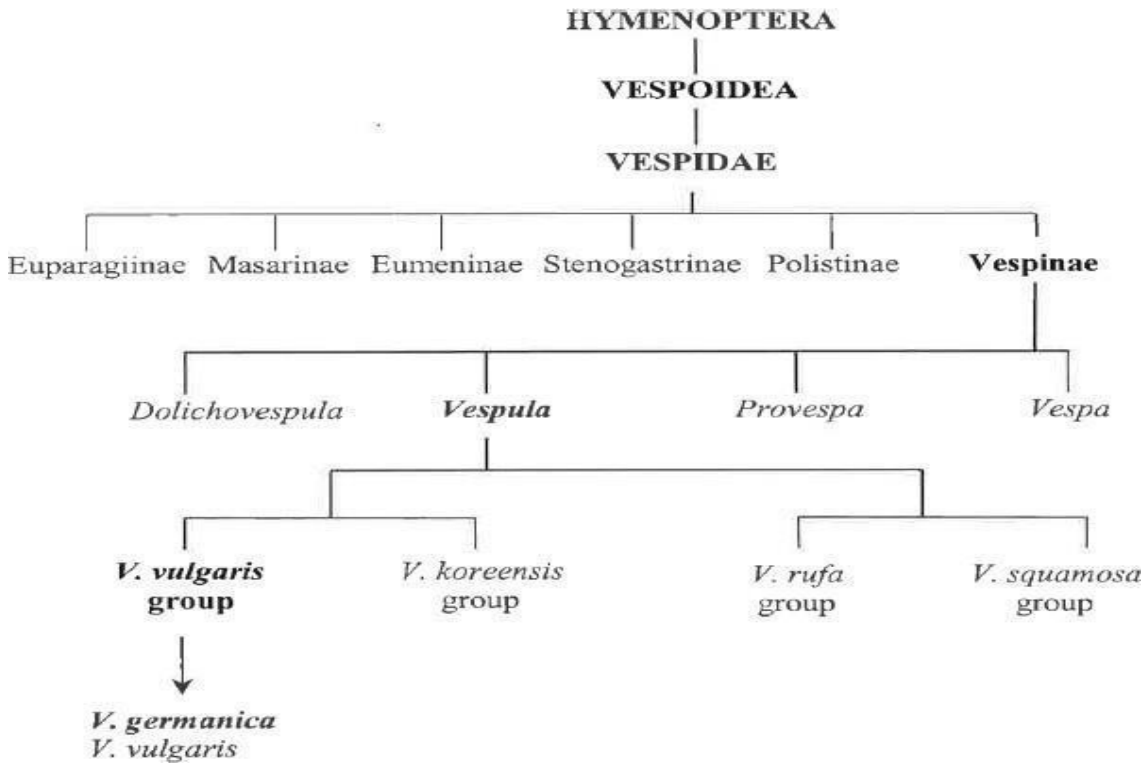

FIGURE 8: Phylogeny of the Vespoidea showing the position of *Vespula germanica* (modified from carpenter 1982 and carpenter 1991).

Table 1: The various levels of sociality found among social insects (from Cowan 1991).

Solitary	Females nest alone and mass provision their nests. They do not interact with their developing young
Presocial	Females exhibit social behaviour beyond sexual interactions, yet short of eusociality
Subsocial	Females nest alone but interact with their developing larvae by progressive provisioning. Females that live sufficiently long may occur on the nest with their adult daughters
Parasocial	Females of the same generation interact on the same nest
Communal	Each female builds, oviposits in, and provisions her own cells
Quasisocial	All females cooperate in building and provisioning brood cells, and all females oviposit
Semisocial	Some females (reproductives) lay most or all of the eggs. Other females (workers) with limited egg- laying opportunities are relegated to foraging, nest building, and caring for the young
Eusocial	Multiple females cooperate in nesting and exhibit reproductive division of labour (asin semisocial), but there is also and overlap of generations, so that adult offspring assist their parents
Primitively eusocial	Colonies are relatively small and short-lived, and morphological differences between reproductive and non-reproductive females are minimal or non-existent
Highly eusocial	: Colonies are relatively large and complex and often are long-lived. Reproductive castes often are morphologically distinct from non-reproductive castes.

II-2 Morphology and social organisation of *V. germanica*

Vespula germanica has a highly eusocial organisation. There are three separate, easily distinguishable, castes present in the colony. These are the relatively rare reproductive males (drones) and reproductive females (gynes and queens), and the numerous sterile females (workers). All adult Vespula, regardless of caste, have a similar morphology. The metasoma is striped black and yellow, and this aposomatic colour patten presumably advertises the presence of a painful sting to their predators. The taxonomy of vespid wasps relies heavily on the size and shape of their colour patterns (Spradbery 1973;Edwards 1980). Figure 8 shows the differences in abdominal markings of V. germanica and V. vulgaris. However, Clapperton et al. (1989b) found a Considerable overlap in markings between the two species in New Zealand as Well as some intermediate patterns, and suggested that other characteristics, Such as markings on other parts of the body, nesting characteristics or Behavioural patterns should also be considered when distinguishing between the species.

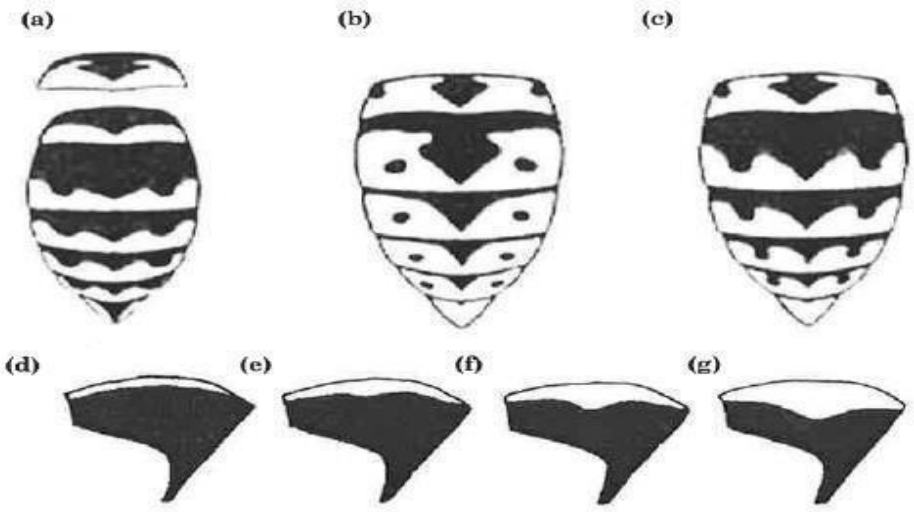

FIGURE 9: The distinguishing dorsal metasomal marks of (a) *vespula vulgaris* and (b and c) *vespula germanica* (from akre et al. 1981), and lateral pronotal markings of (d) vespula vulgaris and (e.f.g) vespula germanica (from clapperton et al. 1989b).

Males and females can be easily separated as males have 8 metasomal and 13 antennal segments, while females have seven metasomal and 12 antennal segments (Spradbery 1973). The queen of V. germanica is the largest caste, measuring 15 to 20 mm in length. The males and workers are smaller,

12 to 13 mm (Edwards 1980), although there is variation in size throughout the season, with the trend being towards larger workers (Potter 1964; Spradbery 1973).

Both reproductive and worker females of Vespula. Possess a sting. Connected to the sting gland, which is usually retracted between the seventh tergile and sternite, and is used for defence (Spradbery 1973). Another gland in the sixth metasomal sternite, the Van der Vecht gland, is thought to produce pheromones, probably for the control of the colony (queen pheromones). In males, the most posterior metasomal segments enclose the genital structure instead of the sting.

Figure 10: *Vespula germanica* (original)

II-3 Determination of sex and caste

One of the characteristics of Hymenoptera, and thus of Vespula, is their haplodiploid method of sex determination. Males develop from unfertilised eggs and are haploid, while females are diploid, carrying maternal as well as patemal genes. Haplodiploidy is thought to be one of the pre-adaptations that enabled the rise of sociality in Hymenoptera (Trivers and Hare 1976). Under this system of sex determination, sisters are related to each other, but only related to their offspring. However, if queens multiply-mate, sister-sister relationships lessen.

Differentiation between worker and queen castes occurs during the fourth larval instar, with queen larvae being fed more than workers (Potter 1964; Spradbery 1973). Male eggs are normally laid by the queen, although worker laid eggs have been detected in some colonies (e.g. Akre and Reed 1983: Ross 1985; Goodisman et al. 2002).

Males and queens are only produced in the nests towards the end of the season. For this purpose, large cells are built. It is not certain what cues are responsible for the onset of large cell production, but

queen age is thought to be involved (Potter 1964).

Table 2: Nest phases of a *Vespula* colony. At the end of the season, the success of the colony is proportional to the position of the phase in the table (from Archer 1980a).

Queen colony	only the queen present before first workers hatch
Small cell colony	Queen and wokers present but no large cells built
Large cell colony	Large cells are present in the colony • large cell colony with eggs • large cell colony with sealed brood adult have emerged : < 100 adults emerged > 100 adults emerged all adults left

II-4 World distribution of *V. germanica and V. vulgaris*

Vespula germanica is native to Europe, northern Africa, the Middle East, northern India, China and Korea, between the latitudes 23°N and 62 N (Edwards 1976, Spradbery and Maywald 1992. Fig. 9) Several species of *Vespula*, including V. vulgaris, are also present in parts of North America. Both *V germanica* and *V. vulgaris* are normally associated with a Mediterranean climate, the hot, dry summers, and cool, wet winters (Edwards 1980) Both are predominantly lowland species (Spradbory 1973), however V. vulgaris has been predominantly lowland species (Spradbery 1973), however V. vulgaris has been shown to exist in sub-alpine environments in its introduced range (Beggs 1991).

Since 1945, both *V. germanica* and *V. vulgaris* have also been introduced to many other parts of the world. They are now established in parts of New Zealand, Ascension Island, South Africa, Chile, Argentina, Canada, U.S.A. and Australia (Edwards 1976; Akre et al. 1989; Spradbery and Maywald 1992; Tribe and Richardson 1994; Vetter et al. 1995; D'Adamo et al. 2002, Fig. 9).

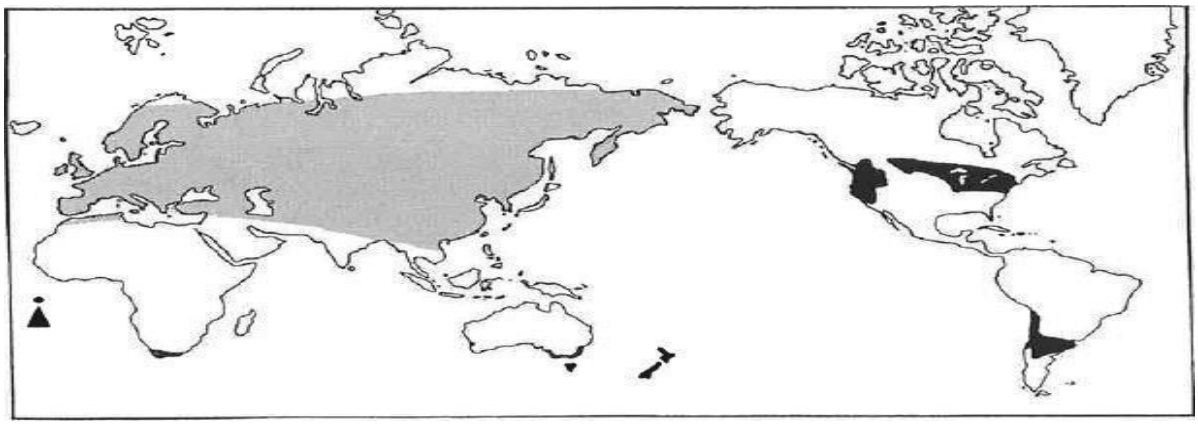

FIGURE 11: The native (shaded) and introduced (black) world distribution of *vespula germanica* (modified from greene 1991). The Ascension island population is marked with an arrow.

II-5 Basic biology and ecology of Vespula

II-5-a Nest site selection and preferences

In their native range in Europe, nests of both *V. germanica* and *V. vulgaris* are found in rural rather than urban areas, and both species prefer nesting in subterranean locations (Spradbery 1973; Pawlikowski 1990). In contrast, in New Zealand, 77% of *V. germanica* nests are found below ground, 20% in aerial sites, and 3% in artificial structures (Donovan et al. 1992). Moller and co- workers (1991a) found that nest site preferences of *V. germanica* and *V. vulgaris* varied between different locations. In beech forests and in horticultural areas 100% of nests were located underground, in urban areas only 60% were subterranean, while 30% were found in artificial structures.

In Australia, Crosland (1991) noted that 42-72% of *V. germanica* nests were found underground, 24-33% were in buildings, and 1-3% were aerial. These figures are not separated into urban and rural areas, and therefore comparisons are difficult. However, it appears that *V. germanica* is less likely to nest underground in Australia than in its native range.

II-5-b Life cycle

The colony life cycle of *Vespula sp*. Is usually annual, with nests being founded by a single queen in spring (Spradbery 1973, see Fig. 10) After emergence from hibernation, the queen searches for a suitable nest site, usually in a dry, dark cavity, and starts building an embryonic nest. The nest is constructed from wood scrapings mixed with salivary excretions and water to produce a brownish or

greyish paper. The colour of the paper varies between species of *Vespula* (Edwards 1980. Akrb of all 1981)

The nest consists of a series of hexagonal cells used for rearing young.arranged in a roughly circular pattern. Each layer of cells forms a comb, and a mature nest in England may comprise up to 15,000 cells and 11 combs (Spradbery 1973; Archer 2001b). Nest construction begins with the attachment of the first layer of 4-5 cells to a buttress (such as a twig) by a spindle. Next, an envelope is built around the nest, presumably for protection from predators and for thermoregulation (Potter 1964). More cells are added to the first comb, and The envelope is also expanded to enclose the whole nest.

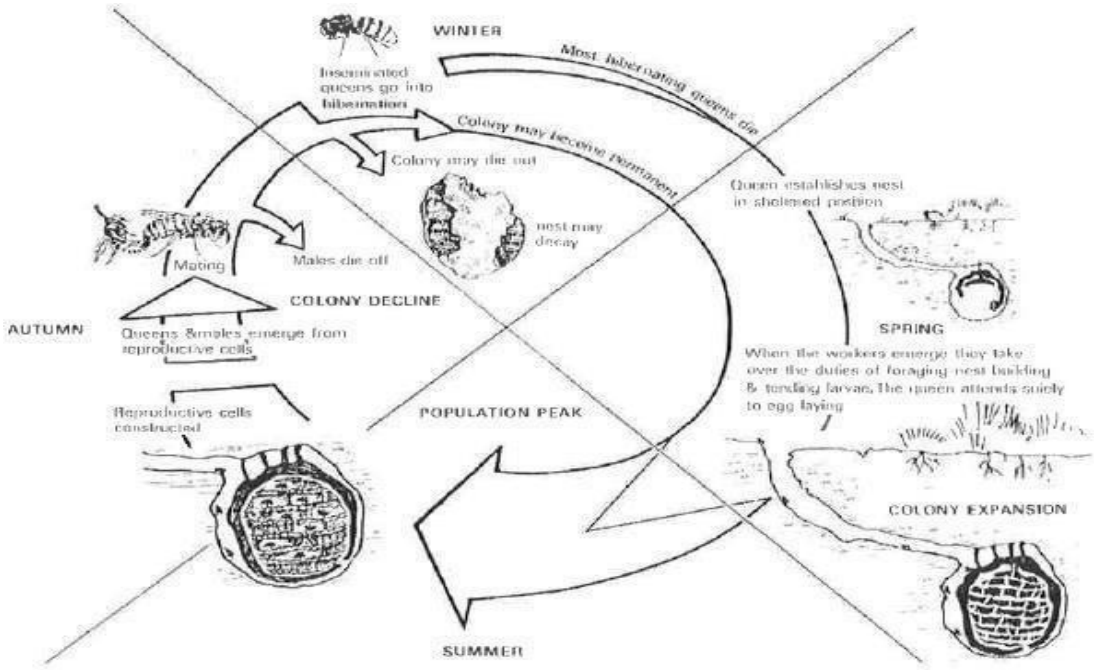

Figure 12: The life cycle of Vespula spp.

The time from establishing the nest until the first workers emerge can be referred to as the 'critical stage at it is a very vulnerable time (Akre and Reed 1981). During this period energy demands on the queen are extremely high, as she has to continue nest building, forage for herself and the developing larvae, as well as protect the nest by herself. Loss of the queen when foraging, or usurpation by other queens is common (Ross et al. 1981; Donovan 1991; Spradbery 1991b; Archer 2001b). Success or failure at this stage is possibly correlated with the fitness of the queen and her ability to protect the nest from usurpations (Leathwick 1997). Queens that have the largest fat reserves, typically found in

successful nests (Harris and Beggs 1995), may be energetically more able to survive the critical period and successfully establish a colony.

After approximately 40 days (for *V. germanica*), the first generation of workers reach adulthood and take over all nest expansion, building, foraging, larva nursing and nest protection activities. The envelope is thickened, and more cells are added. As the nest expands soil is excavated from the bottom of the nest and new layers (combs) are built underneath the old ones (Spradbery 1973). The queen is confined to the nest to continue laying eggs for the rest of the season and her lifetime (Edwards 1980). The workers exhibit a mild form of temporal polyethism, where they start off as nurses, but in time shift to foraging for pulp, prey and liquids, and performing nest guarding duties before dying.

At the end of summer, cells that are 30-40% larger are constructed (Archer 2001b). Most of the males and new queens (gynes) are produced in these large cells, although males can also be produced in small cells. The reproductives disperse and mate, while the drones die off and the nest usually breaks down .The inseminated queens find a dark, dry place and enter reproductive diapause until next spring, when they emerge and start the life cycle again (Spradbery 1973; Edwards 1980; Greene 1991).

Life cycle duration from nest construction to hibernation varies between species of Vespula. In their native ranges it is longest (4 to 5 months) for species in the Vespula vulgaris group (see Fig 7 for classification), and shorter (3 to 4 months) for members of *Vespula rufa* group (Akre et al. 1981).

II-5-c Colony development

The development of each colony may be determined by examination of natural colonies in the field as well as ones reared in the laboratory. Development during the various stages of the life cycle may be described by several characteristics, such as number of cells, brood, adults and meconia, rate of cell-building, production of each brood stage, mortality of each brood stage, and length of life characteristics of the brood, adults and colony (Archer 1997). Further, there are a number of phases the colony can go through, depending on the success of the colony.

There are also some critical times in the development of the colony. These Include queen emergence from hibernation, the emergence of first workers,oundation of the nest, the commencement of large cell production, emergence Of all queens and males, and end of the colony life (Archer 1997). Typically, a mature *V. germanica* colony in England, averages 6,540 small and 1,563 large cells, with the largest colonies producing up to 7,991 workers, 3,215 males and 1,326 queens (Archer 2001b). In

comparison, a V, vulgaris colony, which averages 7,400 small and 2,300 large cells produces 10,293 workers. 1,011 males but only 962 queens (Archer 1980a). This is due to V. vulgaris utilising more of its large cells for male rather than queen production. Comparable data on nest sizes does not exist for New Zealand or Australia, apart from the large overwintered nests. This is urgently needed as it forms the basis of any population ecology studies.

III BEE VENOM

III-1 The inoculating appariel

III-1-a the sting

Only female bees have a sting. The vulnating apparatus, or sting, is actually a modification of the ovipositor,the organ used by parasitic insects to deposit eggs. **(Adam 2010).**

(from the Latin *vulnerare*: to hurt, *vulnus*: wound).

Figure 13: *Apis mellifera's* stinger The vulnating apparatus comprises:

- ✓ Two barbed bristles that form the stinger and slide inside a swollen piece of chitin, the gorgeret.
- ✓ Two sheaths protecting the sting;
- ✓ Venom glands. The acid gland feeds the venom reservoir, the swollen part of the gorgeret, and thealkaline gland helps lubricate the sting.
- ✓ A venom pouch where venom is stored.

- ✓ Chitinous parts and muscles that allow the sting to emerge and thevenom to be injected.

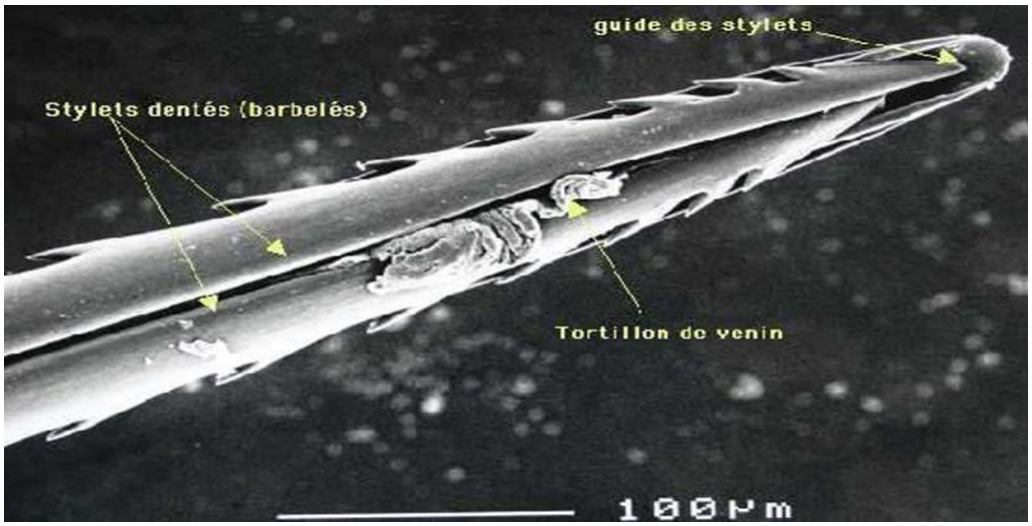

Figure 14: Worker stinger observed under a scanning electron microscope **(Gherbi 2011).**

III-1-b The glandular complex

Venom is a mixture of several compounds, produced by two glands, the venom gland and the lubricating gland, stored in a reservoir. The precise role of the lubricating gland (or Dufour's gland) is still poorly understood. The venom gland, on the other hand, produces a poison so violent that an injection of 0.3 mg subcutaneously in humans is enough to cause very sharp pain. The venom gland is said to be "acidic" and the lubricating gland "alkaline", due to the pH of their secretions.

The venom is therefore a mixture of the products of these two glands, and is channeled through a gutter formed by the juxtaposition of two perforating bristles (or stylets), barbed in the worker (Jean-Prost 2005).

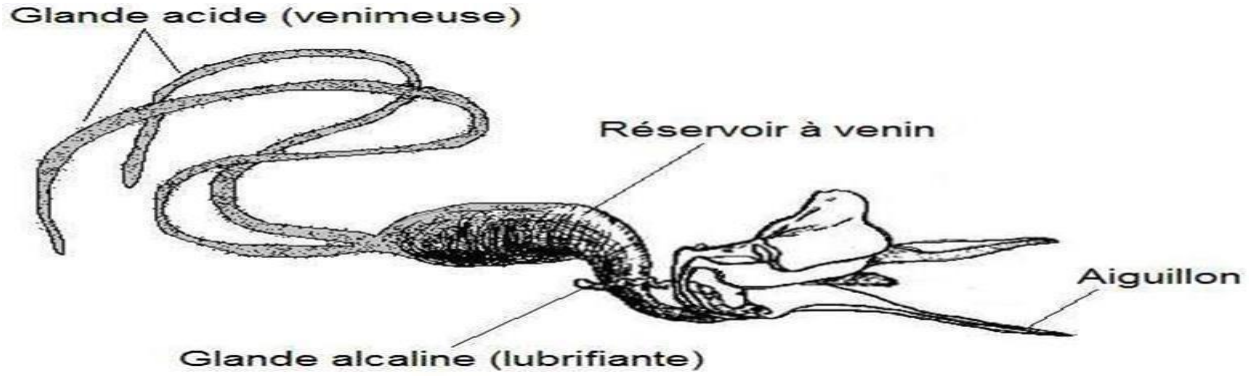

Figure 15: Vulnerating apparatus and associated glands of a worker **(ADAM2010)**

III-2 Venom *harvesting and extraction systems*

The main method for harvesting bee venom is electro-stimulation.

At the entrance to the hive, a thin rubber membrane is placed over which an electric current is diffused when the bee lands on it.

The venom is recovered within a few hours of being exposed to the air, losing some of its volatile compounds **(Alphandéry R. 2002; Libis E. 1971).**

Figure 16: Electro-stimulator used to harvest bee venom.

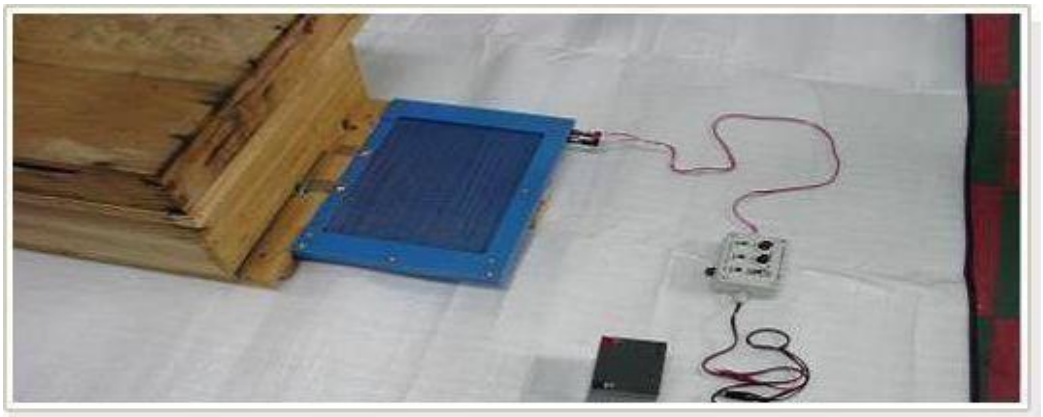

Figure 17: Venom harvesting device

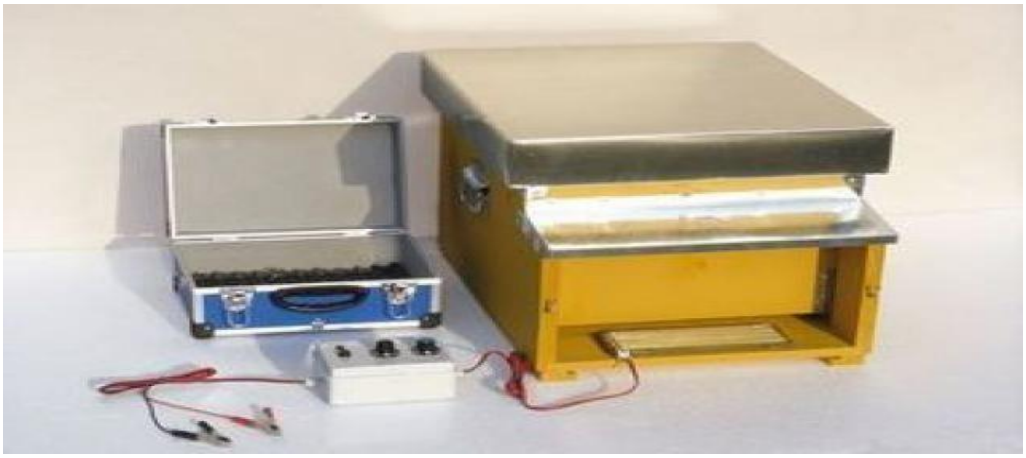

Figure18: PICK-O-TRONIC Electronic bee venom collector

Figure 19: scraping dehydrated venom from glass plate

III-3 Composition of venom

Venom is translucent, liquid and contains numerous peptides, enzymes and amines. Venom composition varies according to 4 parameters: the type of nectar consumed, the type of pollen consumed, the age of the bee and the species **(Apimondia 2001; Cherbuliez 2001a)**. It contains 85% water for 15% dry matter.**(Becker 2010).**

III-3-a Volume

The volumes of venom sacs and their contents in terms of quality and quantity vary according to the species, age and social group of the insect. Venom comprises 90% water and 10% various substances, of which some sixty have been identified. However, there are significant differences between authors (Table 1).

Schematically, venom contains 3 types of substance: biogenic amines with toxic properties, polypeptides and non-enzymatic proteins, which also have toxic properties but some of which are allergens, and enzymes, which comprise the majority of allergens while retaining their functional properties, notably

protease and cytotoxic activity.Over a hundred peptides and proteins have been identified in bee venom .

III-3-b Variations

- depending on extraction techniques (decortication or electro-stimulation): bee venom contains varying levels of enzymes (including hyaluronidase) and mellitine (Figure 18).Batches of venom can therefore vary, with different toxic and allergenic properties.
- At present, allergen extracts are calibrated on major allergens and, above all, on their phospholipase A2 (PLA2) content.
- -Hyaluronidase activity peaks as soon as the bee is born and remains constant throughout its life. Histamine,on the other hand, reaches maximum concentration only around the 35th day after hatching (Figure 19).
- Depending on social rank: workers have a larger venom sac and more abundant protein content; queenshave a 3-fold smaller reservoir and less hyaluronidase. The same applies to PLA2. On the other hand, it contains large quantities of vitellogenin.
- depending on the species: long-standing studies have sought to determine whether venom composition differs between the European honey bee and hybrid African species, which are more aggressive and have been described as "killer bees" in small epidemics in the USA, with higher morbidityrates. Qualitatively, the venoms are similar, notably in terms of allergen and PLA2 content. However, the quantity injected varies 147 micrograms on average for European species, 94 micrograms for Africanspecies. The lethal dose in mice is equivalent, and the epidemiological differences are purely behavioral.

III-3-c Allergens

Two substances have been identified as major allergens in immunoblot studies: PLA2 and hyaluronidase; icarapine (Api m 10) has recently been added. Mellitine is less allergenic. Other allergens are listed, with less significant immunological properties (Table 4). Not all allergens have yet been identified.

- ✓ **Phospholipase A2 (PLA2, Api m 1)**

It accounts for 10-15% of bee venom proteins. It is a small glycoprotein of 134 amino acids, with a molecular weight of 16 to 20 kDa. In international nomenclature, it is coded Api m 1. It has two poles, hydrophilic and hydrophobic, with a calcium-binding site. Its biological properties are similar to those of other phospholipases, including the human phospholipase involved in inflammatory processes. Its structureis known, and the enzyme is present in other venoms, including snake venom

and porcine pancreas, but with little sequence identity (around 20%), which means it is not cross-reactive. The same appliesto phospholipases in vespid venoms, and it is accepted that there is no cross-reactivity between apids and vespids using this enzyme.

It is a major allergen, recognized by 90% of patients allergic to bee venom. The work of King Has shown that PLA2 has 10 times the allergenic activity of total venom, but with a potential that varies from patient to patient. Only exceptionally does it remain less reactogenic than other allergens, including hyaluronidase.

PLA2 has been cloned and is available in recombinant rApi m 1 form for specific IgE titration (immunoCAPi268 allergen component). IgE binding capacity in bee venom-sensitized patients is identical between recombinant and natural proteins.

Api m 1 from different bee species are closely related and Api m 1 also has 53% structural homology with bumblebee venom phospholipase A2.

Api m 1 is an allergen rich in carbohydrate determinants (CCDs), which can cause false-positive in vitro tests by cross-reacting with other CCDs present on other allergens: pollens, latex, foods,other venoms including vespid venom (except polistes venom, which is devoid of CCDs). The recombinant form currentlyavailable does not contain CCD, as it is cloned from Escherichia coli. This improves the specificityof in vitro tests, and Muller's work showed that the sera of 97% of true bee venom allergy sufferers recognized rApi m1. However, sensitivity is lower, ranging from 57% to 82%, and the absence of reactivity to Api m1 remains insufficient to exclude sensitization to bee venom. Other allergens (Apim 2 and Api m 10) must be taken into account.

In addition to its allergenic properties, PLA2 has strong enzymatic and cytotoxic activity. On its own, PLA2 is non-toxic, but it combines with mellitin to form a complex known as bee hemolytic factor, whichcleaves cell membrane phospholipids.

It is responsible for cell damage through hydrolysis of the 2-ester bonds of L-series glycerophospholipids. This enzymatic activity is not necessarily linked to its allergenicity. However, the release of histamine, 5-OH-tryptamine, hexosaminidase and pro-Th2 cytokines, including interleukin 4, in rats follows the action of PLA2 on membrane phospholipids.

Cytotoxic activity remains present in the absence of specific antibodies. PLA2 induces non-specific histamine release by human basophils, and its action on membrane phospholipids makes tissues

more permeable to rapid venom diffusion. It can also induce smooth muscle contraction, hypotension and increased vascular permeability. Recombinant PLA2 has the same enzymatic properties as naturalPLA2, with identical non-specific histaminoliberation phenomena.

A phospholipase B is also known to enhance PLA2 activity. Phospholipases B are important components of snake venom.

- ✓ **Hyaluronidase (Api m 2)**

It is a 43 kDa basic glycoprotein, capable of cleaving hyaluronic acid and increasing connective tissue permeability. It represents around 1% of the venom's dry weight. It plays an important role in venom diffusion and potentiates the activity of other constituents. It is not directly neuro- or cytotoxic, and cleavesthe glycosylated bridges -1,4 between N-acetylglucosamine and D-glucuronic acid.

It is a labile enzyme whose presence is inconsistent in venom batches. It is a useful component for standardizing allergen extracts.

Api m 2 is a major allergen and a recombinant rApi m 2 has been synthesized. Expressed in E. coli, it has a lower enzymatic activity (30%) than the purified natural allergen and a lower IgE-binding capacity.Another recombinant obtained from Baculovirus is glycosylated but has the same enzymatic and antigenic activity as the natural allergen. Here, the molecule's tertiary structure is important for its biological activity.

Its structure is known: it is a globular protein composed of 11 sheets and 10 helices. Bee hyaluronidase has 50% sequence identity with that of vespids, which may explain certain cross-reactivitiesby this allergen, but its clinical relevance remains to be determined.

- ✓ **Mellitine (Api m 4)**

This bee-specific allergen accounts for 50% of venom weight.It is a small polypeptide of 2480 Da and 26 amino acids, involved in pro-inflammatory properties. It is a potent toxicant with surfactant detersive activity by reducing surface tension. Mellitine has considerable hemolytic activity and is cytotoxic. It is thus involved in non-specific histaminoliberation by mast cells and basophils. At the cellular level, it interacts with cell membrane enzyme systems by interrupting the oxidative phosphorylationof nucleotides. It may release chemotactic factors for eosinophils and neutrophils. It potentiates PLA2, whose activity increases 5-fold in the presence of mellitine. It has been

attributed anti-microbial, anti-viral, anti-inflammatory and anti-neoplastic properties in vitro and in vivo.

In the case of bee stings, it is responsible for significant pain through direct and indirect action on nociceptive receptors, and for a persistent inflammatory reaction. It also has a powerful vasodilatory action on capillaries, hypotensive activity and smooth muscle contraction. It is thought to be involved in the toxic side-effects observed with specific immunotherapy, which are more frequent with bee venom thanwith wasp venom.

Its antigenicity is certain, but it is a minor allergen, recognized by 25 to 35% of patients sensitized to bee venom. International nomenclature classifies it as Api m 4; isoforms exist, mellitin-S and mellitin- F, but in small quantities in venom; recombinants are not yet on the market, but its crystalline structure has been published and it is available as a synthetic peptide.

- ✓ **Other allergens**

Bee venom contains an acid phosphatase (Api m 3), a 49 kDa glycoprotein of which 70% of the gene has been cloned and sequenced. This allergen belongs to the family of acid phosphatases of lysosomal origin. Acid phosphatase is also present in the venom of vespids. Epidemiological studies have shown that it is an allergen recognized by 37-60% of patients sensitized to bee venom. The recombinant form is less reactive than the natural form, recognized by only 37% of allergic patients. Acid phosphatase is still considered a minor allergen. Its role in cross-reactions between venoms is uncertain. Like hyaluronidase, it has toxic activity and promotes venom diffusion. Its under-representation in allergenic extracts could explain some false-negative results.

We also know of a protease-active enzyme with a molecular weight of 39 kDa, which is probably a major allergen, called Api m 7 , and a polypeptide with 71 amino acids and a molecular weight of 7.9 kDa, Api m 6, a minor allergen corresponding to 1 to 2% of the dry weight of venom . It is recognized by 42% of patients. It exists in 4 isoforms from 7190 to 7808 Da. No relationship with known proteins could be determined during sequencing. As Api m 6 is not glycosylated, its functional properties remain unknown.

A high-molecular-weight (102 kDa) C allergen has been identified as a dipeptidylpeptidase (Api m 5) by Blank. This minor allergen is close to the dipeptidylpeptidases of the wasp Vespula Ves v 3 and the wasp Polistes Pol d 3, with cross-reactions occurring in over 50% of cases. An IgE assay for Api m 5 is available for routine diagnosis.

A carboxylesterase (Api m 8) and a carboxypeptidase (Api m 9) have also been isolated and recognizedas minor allergens.

Icarapine (Api m 10), a carbohydrate-rich peptide present in the venom duct lining, has been recognized by IgEs from some beekeepers and then from allergic patients. It is an unstable allergen of unknown function with at least 9 isoforms. Icarapin-like proteins have been identified in numerous insect species: in the Hymenoptera order, in Apis cerana bees and the solitary cutter bee Megachile rotundata, bumblebees (Bombus terrestris), wasps (Polistes dominula), and ants (Solenopsis invicta). It is also present in beetles (Leptinotarsa decemlineata), flies (Drosophila grimshavi), fleas, termites, mosquitoes, thrips andbeetles. However, the only insect in which an Api m 10 homologue has been identified as a venom component by proteomic techniques is the wasp Polistes dominula. This is a major allergen with numerousdiagnostic and therapeutic implications. It is a marker of bee venom allergy useful in differential diagnosis, but its under-representation in certain extracts for therapeutic use could explain failures in allergen immunotherapy.

Also isolated as allergens from royal jelly proteins are Major Royal Jelly Protein 8 (MRJP 8) and Major Royal Jelly Protein 9 (MRJP 9). These are glycosylated components giving false IgE-reactivity via thecarbohydrate determinants CCD, but their allergenicity has been demonstrated by skin tests in 15% of patients allergic to bee venom for MRJP 8 and 34% for MRJP 9. This reactivity is close to that of Apim 2, making them a minor allergen to be considered. The international codes for these allergens are Api m11.0101 and Api m 11.0201.

The allergenic role of vitellogenin, whose nomenclature is Api m 12, has also been demonstrated. The precursor of vitellogenin is a lipoglycoprotein synthesized and stored in fat, then secreted in the hemolymph in mature form. In bees, vitellogenin is most abundant in the queen bee, and after synthesis is transportedto the oocytes where it nourishes the embryos. It plays an important role in hormone signaling, the transitionfrom nurse to forager bee, stress resistance and longevity in worker bees. A vitellogenin is also presentin the wasp Vespula with the same functions and comparable allergenicity (Ves v 6). These 200 kDa proteins have a structural homology of 40%, making them potential cross-allergens. Vitellogenins have been synthesized as recombinants for both bees and Vespula. These allergens are recognized by 40% of sera from allergic patients after elimination of carbohydrate determinants. They are minor allergens with allergenic potential in certain circumstances, and should be considered when investigating cross- reactions between bees and Vespula.

III-3-d Biogenic amines, polypeptides and non-allergenic proteins

A large number of substances with powerful pharmacological properties but no immunogenic role have been identified in bee venom. These substances may contribute to the toxic action of the venom and the spread of allergens through their inflammatory properties (Tables 4 and 5).

- ✓ **Biogenic amines**

Histamine, noradrenaline and dopamine are present in bee venom.Up to 2 µg of histamine have been collected in a worker venom bag. These amines are responsible for the classic vasomotor phenomena observed in the non-specific envenomation reaction.

- ✓ **Peptides**

Apamin is specific to bee venom. It consists of 18 amino acids and has a molecular weight of 2027 Da. It is a basic peptide (2% of dry weight) with peripheral and central neurotoxic properties via the meningeal barrier. It blocks potassium-dependent calcium channels in the central nervous system (SK2 andSK3 channels), which implicates it in neurotoxic effects after stings or during specific immunotherapy withbee venom. It is also a vasoactive intestinal polypeptide antagonist of non-adrenergic, non-cholinergic intestinal dilatation, and an antagonist of smooth muscle relaxation.

The mast-cell degranulating peptide (MCD) is a small molecule of 2590 Da (1% of dry weight) which produces non-specific degranulation of mast cells. It is also present in the venom of vespids. It is a powerful histaminoliberator, more potent than mellitine. Induced histaminoliberation is responsible for vasodilation, venom diffusion and inflammatory reactions.

III-3-e Enzymes and other substances

In addition to allergens with enzymatic properties, bee venom also contains glucidases, lipases and an N- gly-pro-aryl-amidase. These enzymes contribute to the venom's cytotoxicity, toxic action and diffusion. The same enzymes are present in other venoms (snakes, fish, invertebrates).

This list is not exhaustive, as we also know cardiopep, a peptide with tachycardia-inducing activity, and substances with pharmacological activity that is still poorly understood, such as secapine, tertiapine, procamine and osmine, a peptide with antimicrobial activity close to MCD present in the solitary bee Osmiarufa.

There are also various acids, hydrochloric acid, formic acid, phosphoric acid... and undoubtedly

other substances yet to be discovered.

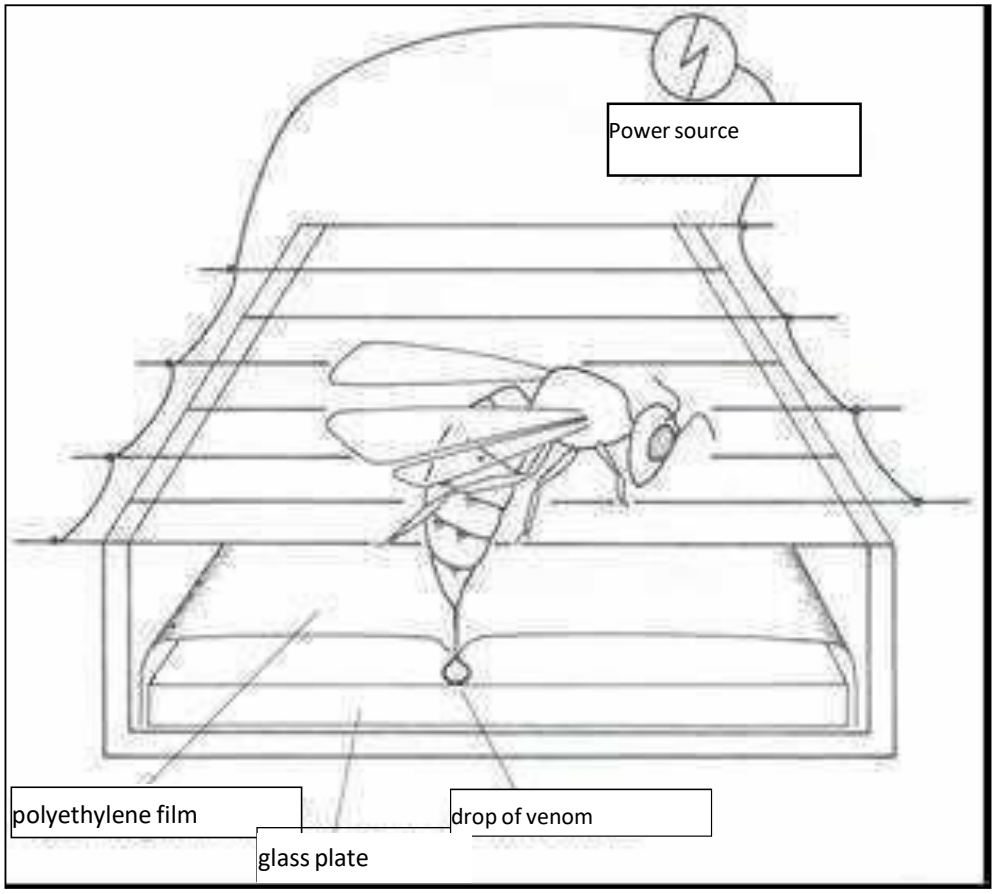

Figure 20: Collection of bee venom by electro-stimulation (Document Stallergènes Greer)

CHAPTER II: MATERIALS AND METHODS

CHAPTER II : MATERIALS AND METHODS

I Presentation of biological material

I-1 Vespula germanica

The study was conducted on the vespula germanica species. For our identification we used the following guides :

- Key to the paper and social wasps of Central Europe (Hymenoptera: Vespidae) by Libor DVOŘÁK[1)] & Stuart P. M. ROBERTS[2)] 2006
- Identification des guêpes sociales (Vespa, Vespula, Dolichovespula ; Vespidae : Vespinae) de France, femelles uniquement (Jérôme CARMINATI) 2020.

Figure 21: *Captured Vespula germanica* (original)

I-2 Experiment boxes

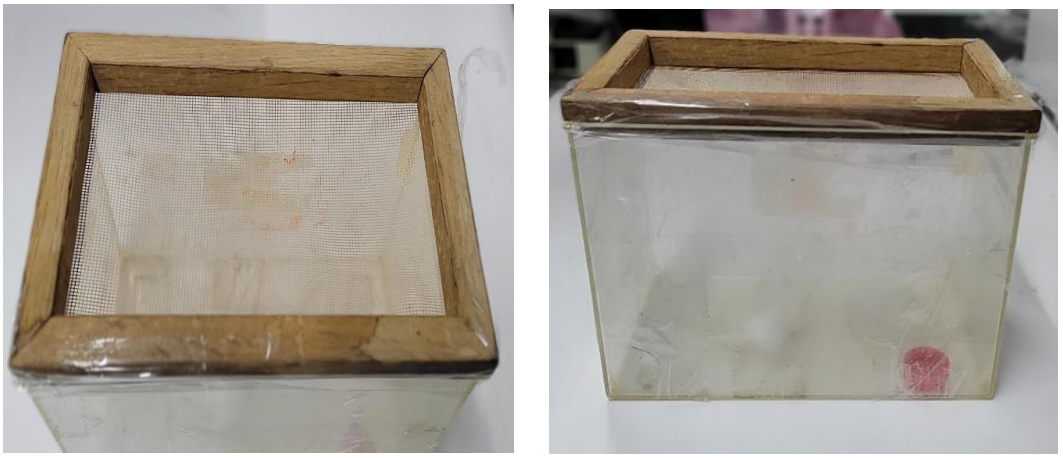

Figure 22: *photos of experiment boxes* (original)

CHAPTER II : MATERIALS AND METHODS

II Treatment of insects

In the treatment of insects, specifically *Vespula germanica* wasps, several materials were utilized.

Firstly, a total of 18 mg of natural bee venom was employed for the experiment. To dissolve the bee venom, 180 ml of distilled water was used, ensuring the purity of the venom solution.

To facilitate the experiment, four plastic boxes were employed. Each of these plastic boxes was equipped with ventilation holes to maintain adequate airflow, which is crucial for the well-being of the wasps throughout the experiment.

To provide sustenance for the wasps during the experiment, various food sources were provided. Dead insects and small pieces of banana were chosen as suitable food options, along with water. For the application of the venom, a micropipette with a range of 1-20 ul was used. This micropipette allowed precise and controlled administration of the venom to the wasps.

Following the protocol described by Delobel et al. (1998), the 24 *Vespula germanica* wasps were divided into four groups, with six individuals in each group. This division allowed for the comparison of different venom dosages on the toxicity response of the wasps.

One of the groups served as the control group, receiving no venom application. The control group acted as a baseline for comparison against the experimental groups, helping to evaluate the specific effects of the venom. In the second group, 24 µl of bee venom solution was applied per wasp using the micropipette (resulting in 144 ul of venom in the second group's box). In the third group, each insect was administered 36 µl of venom (totaling 216 ul in the third group's box), while in the fourth group, 54 µl of venom per insect was used (amounting to 324 ul in the fourth group's box). These varying dosages aimed to assess the dose-dependent effects of the venom on *Vespula germanica*.

To ensure controlled and localized exposure to the venom, the bee venom was applied to a piece of cotton in each glass box. This method allowed for consistent delivery and prevented unintentional exposure or contamination.

Throughout the experiment, the glass boxes were maintained under suitable environmental conditions. Adequate ventilation was provided to ensure proper airflow. The temperature was controlled, with the maximum being 27°C and the minimum being 13°C. Additionally, the humidity levels were maintained between 45% and 70% to ensure the overall well-being of the wasps.

CHAPTER III: RESULTS

CHAPTER III: RESULTS

I Mortality post treatment

The following tables shows the results of our tests of mortality post treatment of *Vespula germanica* in each group with bee venom

Table 3: the mortality rate of *Vespula germanica* post treatment with natural *Apis mellifera's venom*

Doses (µl/insect)	**Number of the tested insects**	**%Mortality post treatment**													
		0h	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Group1: 24	6	0 %	0%	0%	0%	16 %	16 %	33 %	50 %	66 %	100 %	100 %	100 %	100 %	100 %	100 %
Group2: 36	6	0 %	0%	16 %	16 %	33 %	50 %	66 %	83 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
Group3: 54	6	0 %	0%	33 %	66 %	83 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
Group4: Control Group	6	0 %	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	16 %	33 %	83 %	100 %

This table represents the results of an experiment conducted to study the effects of different doses of bee venom on *Vespula germanica* insect mortality over a specified time period.

In the experiment, four groups were tested, each receiving a specific dose of bee venom. The doses used were 24 µl/insect for Group 1, 36 µl/insect for Group 2, and 54 µl/insect for Group 3. The control group did not receive any treatment with bee venom. The insects used in the experiment were *Vespula germanica*.

For Group 1, which received a dose of 24 µl/insect, *Vespula germanica* insects tested. At 0 hours and 1 hour post-treatment, no mortality was observed. However, starting from 2 hours, there was a gradual increase in mortality percentages: 16% at 4 hours, 16% at 5 hours, 33% at 6 hours, 50% at 7 hours, 66% at 8 hours, and 100% from 12 hours onwards. This means that all *Vespula germanica* insects in Group 1 treated with a dose of 24 µl/insect were deceased by the 12-hour mark and remained so throughout the duration of the experiment.

Group 2, which received a dose of 36 µl/insect, Similar to Group 1, no mortality was observed at 0 hours and 1 hour post-treatment. However, the mortality percentages increased over time: 16%

at 2 hours, 16% at 3 hours, 33% at 4 hours, 50% at 5 hours, 66% at 6 hours, 83% at 7 hours, and reached 100% from 8 hours onwards. This indicates that all *Vespula germanica* insects in Group 2 treated with a dose of 36 µl/insect were deceased by the 8-hour mark and remained so throughout the experiment.

Group 3, which received the highest dose of 54 µl/insect, After 0 hours and 1 hour post-treatment with bee venom, no mortality was observed. However, the mortality percentages increased more rapidly compared to the other groups: 33% at 2 hours, 66% at 3 hours, 83% at 4 hours, and reached 100% from 5 hours onwards. Therefore, all *Vespula germanica* insects in Group 3 treated with a dose of 54 µl/insect were deceased by the 5-hour mark and remained so throughout the duration of the experiment.

In contrast, the control group, labeled as Group 4, did not receive any treatment with bee venom. At all time intervals up to 24 hours, 0% mortality was observed. However, at 48 hours, there was a 16% mortality rate, which increased to 33% at 72 hours, 83% at 96 hours, and reached 100% at 120 hours and subsequent time intervals. This indicates that natural mortality occurred in the control group, but it took longer for all *Vespula germanica* insects to perish compared to the treated groups.

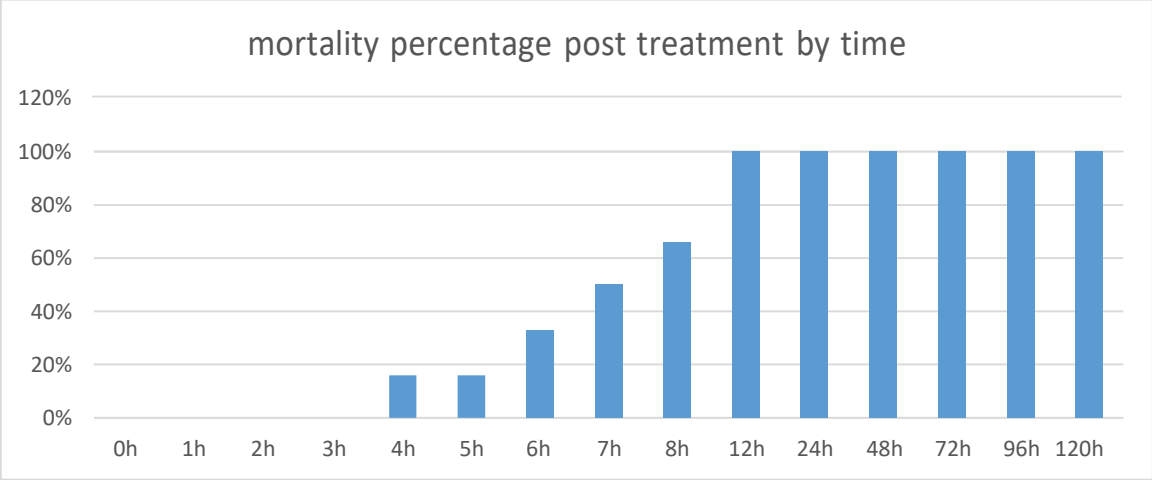

FIGURE 23: Histogram of group 1 (mortality post treatment by time)

The histogram titled "Mortality Rate of *Vespula germanica* Post-Treatment with Natural *Apis mellifera's* Venom (Group 1)" displays the relationship between the dose of venom administered and the corresponding mortality rate over a period of time. The dose of the venom used in this study is 24, and the mortality rate is measured at various time intervals following the treatment, starting from 0 hours.

At the beginning of the experiment, immediately after the treatment (0 hours), the mortality rate

was recorded as 0%. This indicates that no mortality occurred immediately after the administration of the venom. Similarly, after 1 hour, 2 hours, and 3 hours, the mortality rate remained at 0%, indicating no observable deaths within this timeframe.

However, at the 4-hour mark, the mortality rate increased to 16%. This suggests that 16% of the treated *Vespula germanica* specimens experienced mortality four hours after the venom was administered. The same mortality rate of 16% was observed after 5 hours as well.

As time progressed, the mortality rate continued to rise. At the 6-hour mark, the mortality rate increased to 33%, indicating a higher number of deaths among the treated specimens. Subsequently, at 7 hours and 8 hours, the mortality rates further increased to 50% and 66% respectively, indicating a significant impact of the venom on the survival of *Vespula germanica.*

The highest mortality rate of 100% was observed after 12 hours and remained at 100% until the end of the experiment at 120 hours. This indicates that all the Vespula germanica specimens treated with the given dose of *Apis mellifera's* venom had succumbed to mortality by the end of the observation period.

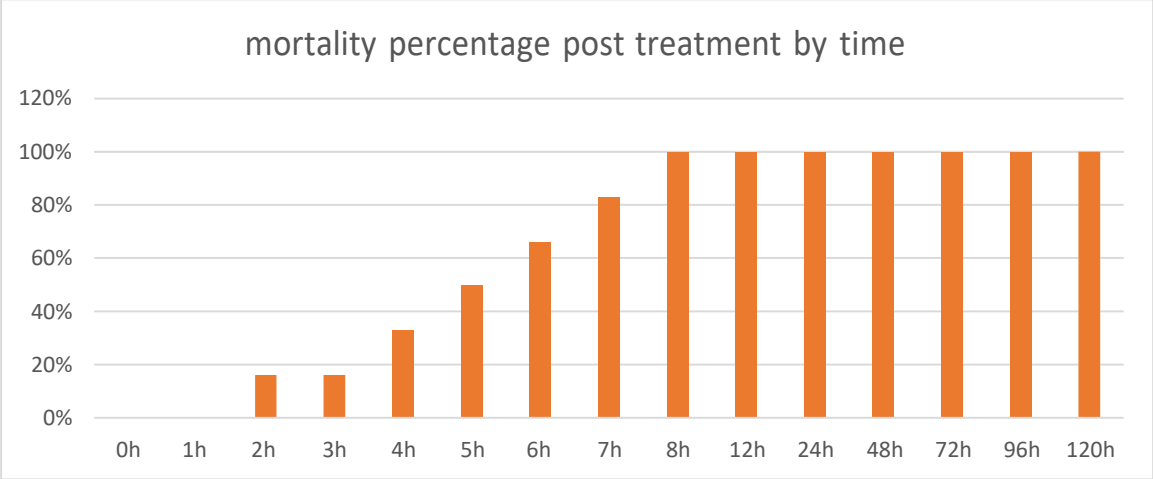

FIGURE 24: Histogram of group 2 (mortality post treatment by time)

The histogram titled "Mortality Rate of *Vespula germanica* Post-Treatment with *Natural Apis mellifera*'s Venom (Group 2)" displays the relationship between the dose of venom administered and the corresponding mortality rate over a period of time. The dose of the venom used in this study is 36, and the mortality rate is measured at various time intervals following the treatment, starting from 0 hours.

At the beginning of the experiment (0 hours), the mortality rate was recorded as 0%. This suggests that no immediate mortality occurred immediately after the treatment with *Apis mellifera's* venom.

Similarly, after 1 hour, the mortality rate remained at 0%, indicating no observable deaths within this timeframe.

However, at the 2-hour mark, the mortality rate increased to 16%. This implies that approximately 16% of the treated *Vespula germanica* specimens experienced mortality after two hours of treatment. The same mortality rate of 16% was also observed after 3 hours, indicating a consistent level of mortality within this period.

As time progressed, the mortality rate continued to rise. At the 4-hour mark, the mortality rate increased to 33%, indicating that approximately one-third of the treated specimens had succumbed to mortality by this time. After 5 hours, the mortality rate further increased to 50%, suggesting a significant rise in deaths compared to the previous time interval.

By the 6-hour mark, the mortality rate reached 66%, indicating that approximately two-thirds of the treated *Vespula germanica* specimens had died. This trend continued with the mortality rate increasing to 83% after 7 hours and further to 100% after 8 hours.

The mortality rate remained at 100% for the subsequent time intervals of 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours. This indicates that all the *Vespula germanica* specimens treated with *Apis mellifera's* venom in Group 1 experienced mortality and did not show any signs of survival throughout the entire duration of the observation period.

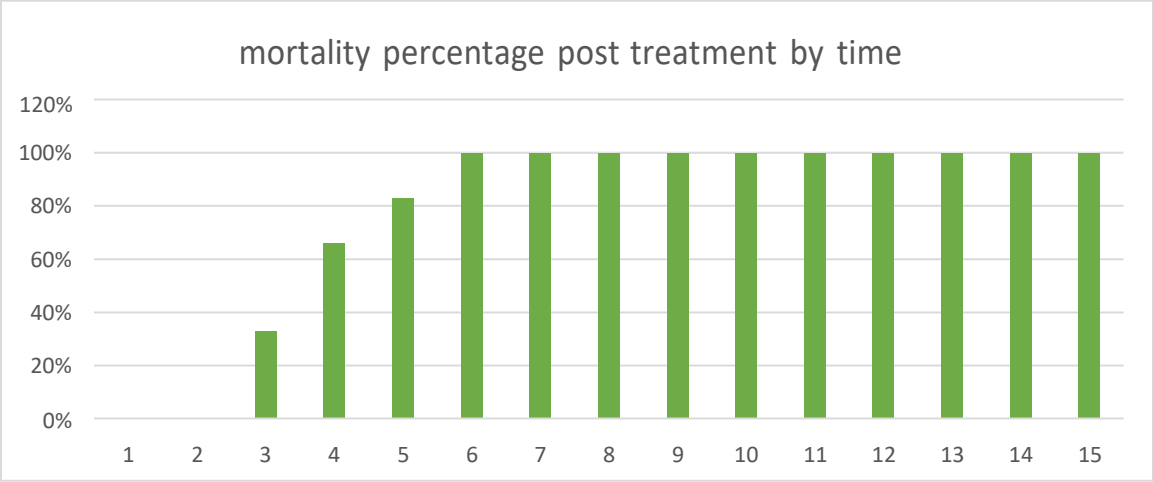

FIGURE 25: Histogram of group 3 (mortality percentage post treatment by time)

The histogram titled "Mortality Rate of *Vespula germanica* Post-Treatment with *Natural Apis mellifera*'s Venom (Group 3)" displays the relationship between the dose of venom administered and the corresponding mortality rate over a period of time. The dose of the venom used in this study is 54, and the mortality rate is measured at various time intervals following the treatment,

starting from 0 hours.

At the beginning of the experiment (0 hours), the mortality rate was recorded as 0%. This indicates that no immediate mortality occurred following the treatment with *Apis mellifera's* venom. Similarly, after 1 hour and 2 hours, the mortality rate remained at 0%, suggesting no observable deaths within these timeframes.

However, after 3 hours, the mortality rate increased to 33%, indicating that approximately one-third of the treated *Vespula germanica* specimens experienced mortality. As time progressed, the mortality rate continued to rise.

After 4 hours, the mortality rate increased to 66%, indicating a significant increase in mortality compared to the previous time interval. This suggests that a majority of the treated specimens had succumbed to mortality by this point.

By the 5-hour mark, the mortality rate further increased to 83%, indicating a higher proportion of deaths among the treated *Vespula germanica* specimens. The mortality rate reached 100% at the 6-hour mark, indicating that all the treated specimens had died by this time.

The mortality rate remained at 100% for the subsequent time intervals of 7 hours, 8 hours, 12 hours, and continued until the end of the experiment at 120 hours. This implies that all *the Vespula germanica* specimens treated with *Apis mellifera's* venom in Group 1 experienced mortality and did not show any signs of survival throughout the entire duration of the observation period.

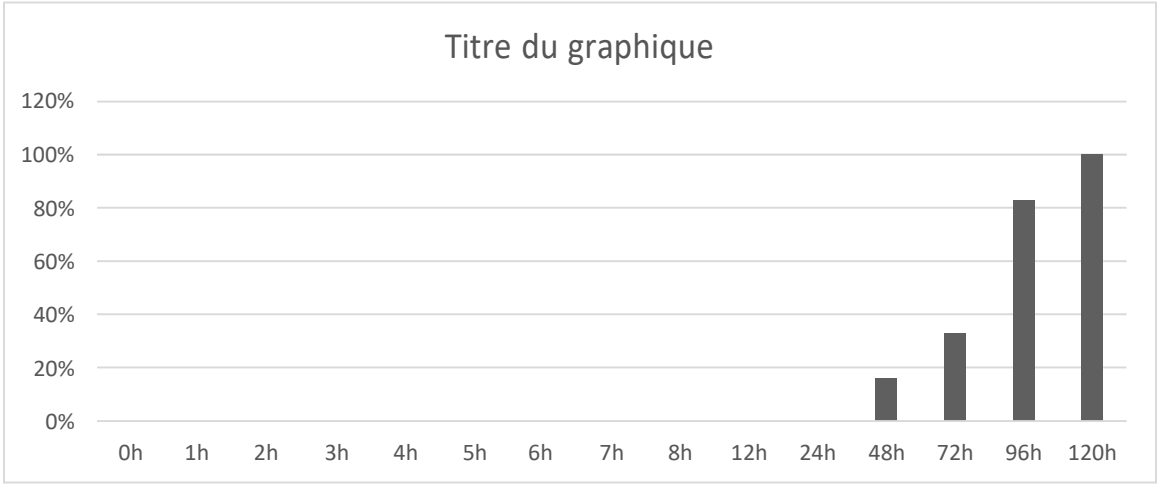

FIGURE 26: Histogram of group 4 (mortality percentage post treatment by time)

The histogram titled "Mortality Rate of *Vespula germanica* in Control Group" visualizes the relationship between the time elapsed after treatment and the corresponding mortality rate. The

data is recorded at various time intervals, starting from 0 hours and extending up to 120 hours.

In the control group, where no venom was applied, the mortality rate was recorded as 0% throughout the initial time intervals. This indicates that there were no immediate deaths observed among the *Vespula germanica* specimens at 0 hours, 1 hour, 2 hours, and 3 hours. The absence of mortality within these timeframes suggests that factors other than the venom did not contribute to the death of the specimens.

As time progressed, the mortality rate remained at 0% up to the 4-hour mark, indicating no deaths during this period. Similarly, there were no recorded deaths at the 5-hour, 6-hour, and 7-hour marks, maintaining the mortality rate at 0% within these intervals as well.

By the 8-hour mark, the mortality rate remained at 0%, indicating that no deaths had occurred even after 8 hours in the control group. This trend continued with the mortality rate remaining at 0% for the subsequent time intervals of 12 hours, 24 hours, and 48 hours.

However, at the 72-hour mark, the mortality rate increased to 16%, suggesting that approximately 16% of the *Vespula germanica* specimens in the control group had experienced mortality after 72 hours. As time progressed, the mortality rate further rose to 33% at 96 hours and eventually reached 100% at the 120-hour mark.

II Statistical analysis

FIGURE 27: Histogram of insects survival by different doses (µl) of bee venom.

The figure 28 shows the viability of *Vespa germanica* individuals after using bee venom in the different volumes of our experiment. We note that at T0 with the control dose, all individuals are

viable, even after the first 48 hours. With the 24µl dose, the first mortality occurred before the first 24 hours and there were no viable individuals after 24 hours. With the last two doses of 36 and 54µl, there was no resistance from individuals after the first 12 hours of the experiments.

III Linear regression

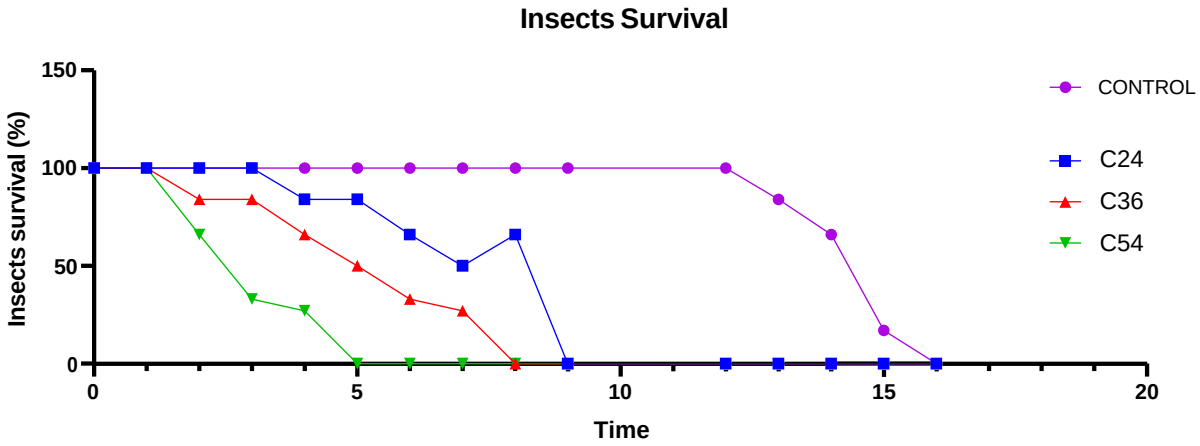

FIGURE 28: Linear regression the viability rate of individuals by time

The figure 29 shows that the viability rate of individuals decreases as a function of time for all four experiments. In fact, for the control test, the rate of individuals begins to decline after 24 h and becomes zero after 12 h, in contrast to the 24, 36 and 54 µl doses, where the rate declines rapidly from the first hour until it becomes zero after 24 h, 12 h and 8 h respectively.

CHAPTER IV:

Discussion and Conclusion

CHAPTER IV: Discussion and Conclusion

Discussion

The present study investigated the effects of bee venom on the mortality of *Vespula germanica,* with a focus on understanding the dose-dependent response and comparing the findings to the results reported in the article by Mamdouh I. Nassar (2013).

The study involved four experimental groups, each receiving a different dose of bee venom, and a control group where no venom was applied. The mortality rates were recorded at multiple time intervals ranging from 0 to 120 hours post-treatment.

The results of the Vespula experiment demonstrated a clear dose-dependent effect of bee venom on the mortality of *Vespula germanica*. As the dose increased, the mortality rates also increased over time. Group 1, which received the lowest dose (24 µl/insect), showed a gradual increase in mortality, reaching 100% at 12 hours post-treatment. Group 2, with a slightly higher dose (36 µl/insect), exhibited a similar pattern, reaching 100% mortality at 8 hours. Group 3, receiving the highest dose (54 µl/insect), displayed a more rapid increase in mortality, reaching 100% within 6 hours. These findings align with the observations in the article by Jerome et al. (2001), where higher doses of bee venom resulted in a higher percentage of mortality in *Sitophilus granarius* weevils.

Comparing the results of the Vespula experiment to the article, we observe similarities in the patterns of mortality rates. In the article, the highest dose of bee venom (6.3µg/insect) caused 94.3% mortality after 72 hours post-treatment, while the lowest dose (1.1µg/insect) resulted in 13.1% and 20.2% mortality rates at 24 and 72 hours, respectively. These findings closely resemble the mortality rates observed in Group 3 and Group 1 of the Vespula experiment. Both studies indicate that higher doses of bee venom lead to more significant mortality rates in the targeted insect species.

Additionally, the control groups in both the Vespula experiment and the article showed no mortality throughout the observation period until reaching 100% at the end. This indicates that the observed mortality in the experimental groups was directly associated with the application of bee venom.

The comparison between the Vespula experiment and the article suggests a consistent dose-dependent response to bee venom across different insect species. These findings support the notion

that bee venom possesses insecticidal properties and can be potentially used as a natural insecticide. The venom's toxic protein and peptide components may be responsible for the observed mortality in *Vespula germanica* and *Sitophilus granarius* weevils. The article also highlights the insecticidal activity of bee venom against other insect species, such as crickets, further emphasizing its potential as an effective biopesticide.

However, it is essential to consider the limitations and variations in the experimental conditions between the Vespula experiment and the article. Each study focused on different insect species, and factors such as the mode of venom application, environmental conditions, and insect physiological differences may influence the observed results. Further research is necessary to explore the long-term effects, ecological implications, and practical applications of bee venom as a pest control agent.

Lastly , the results of the Vespula experiment and the findings reported in the article by Mamdouh I. Nassar (2013).provide valuable insights into the dose-dependent effects of bee venom on insectmortality. Both studies demonstrate the potential of bee venom as an effective insecticide, with higher doses leading to higher mortality rates. These results contribute to the understanding of natural alternatives for pest control and open avenues for further research in this field.

Thesis Conclusion: Utilizing Bee Venom (*Apis mellifera*) as a Control Agent against Parasitic Vespid (*Vespula germanica*)

Conclusion

In conclusion, this study has examined the potential of bee venom derived from *Apis mellifera* as a control agent against the parasitic vespid species *Vespula germanica*. The findings presented and provided compelling evidence that bee venom offers a promising and environmentally sustainable solution for managing and mitigating the negative impacts of vespid populations.

The analysis of this study has shed light on several key aspects that support the feasibility and effectiveness of bee venom as a control agent.

Firstly, the composition of bee venom, characterized by a diverse array of bioactive compounds such as peptides, enzymes, and amines, These bioactive components have the potential to disrupt the life cycle and overall survival.

CHAPTER IV: Discussion and Conclusion

Moreover, it have underscored the selectivity of bee venom, which primarily targets vespid species while posing minimal risks to non-target organisms, including humans. This selectivity mitigates the potential harm to beneficial insects and the wider ecosystem, thereby promoting a balanced ecological system.

Nevertheless, it is crucial to acknowledge the limitations and challenges associated with the utilization of bee venom as a control agent. As evidenced in previous discussions, standardized production methods, dosage optimization, and application techniques require further research and development. Addressing these aspects will be pivotal in ensuring the efficacy and practicality of bee venom-based interventions.

Finally, the findings of this study suggest that the utilization of bee venom derived from *Apis mellifera* holds significant promise as a control agent against parasitic vespid species, particularly *Vespula germanica.* By harnessing the diverse bioactive compounds within bee venom, targeted and sustainable approaches can be developed to effectively manage vespid populations. However, it is imperative that additional scientific investigation and practical implementation be undertaken to fully unlock the potential of bee venom as an efficient and environmentally friendly solution in vespid control strategies.

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APPENDICES

APPENDIX 1: the mortality rate of *Vespula germanica* post treatment with natural *Apis mellifera's venom of group 1.*

Doses µl/insect	**Number of the tested insects**	**%mortality post treatment**													
		0h	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Group1: 24	6	0%	0%	0%	0%	16 %	16 %	33 %	50 %	66 %	100 %	100 %	100 %	100 %	100 %	100 %

APPENDIX 2: the mortality rate of *Vespula germanica* post treatment with natural *Apis mellifera's venom of group 2.*

Doses µl/insect	**Number of the tested insects**	**%mortality post treatment**													
		0h	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Group2: 36	6	0%	0%	16 %	16 %	33 %	50 %	66 %	83 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %

APPENDIX 3: the mortality rate of *Vespula germanica* post treatment with natural *Apis mellifera's venom of group 3.*

Doses µl/insect	**Number of the tested insects**	**%mortality post treatment**													
		0h	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Group3: 54	6	0%	0%	33 %	66 %	83 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %

APPENDIX 4: the mortality rate of *Vespula germanica* post treatment with natural *Apis mellifera's venom of group 4.*

Doses µl/insect	**Number of the tested insects**	**%mortality post treatment**													
		0h	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
Group4: Control Group	6	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	16 %	33 %	83 %	100 %

APPENDIX 5: Volume of venom sacs of different Hymenoptera after David B et al.

	Venom volume (µl)		Dryweight of protein (µg)	
Species	Per bag	By injection	Per bag	By injection
Bee *A. mellifera*	0.6-3	0.5-2	100 72 ±12	70 ± 30 59

APPENDIX 6: Hymenoptera venom allergens and nomenclature.

Apis : Major Royal Jelly Protein.

Venom	Allergen	Name/Function	Molecular weight	Major/ Minor
Apis mellifera	Api m 1	Phospholipase A2	16	Major
	Api m 2	Hyaluronidase	43	Major
	Api m 3	Acid phosphatase	49	Major?
	Api m 4	Mellitine	2.9	Minor
	Api m 5	Dipeptidyl peptidase (Allergen C)	102	Minor
	Api m 6	protease inhibitor	7.9	Minor
	Api m 7	CUB serine protease	39	Major?
	Api m 8	Carboxylesterase	70	Minor
	Api m 9	Carboxypeptidase	60	Minor
	Api m 10	Icarapine	24.8	Major
	Api m 11.0101	MRJP 8 (major royal jelly protein)	65	Minor
	Api m 11.0201	MRJP 9 (major royal jelly protein)	50	Minor
	Api m 12	Vitellogenin	200	Minor

APPENDIX 7: Pharmacological properties of bee ve no m constituents. According to Habermann.

	Histamine	Mellitine	Apamine	MCD Peptide	Hyaluronidase	Phospholipase A 2
Dry weight (%)	0.1-1	50	2	2	1-3	12
Molecular Weight	111	2840	2038	2593	> 20000	524
Pain	++	++	?	?	-	?
Vasodilation	++	++	+	+	+	+
Cytotoxicity	-	++	?	+	-	+
Neurotoxicity	-	+	++	+	-	-
Hemolysis Direct	-	++	+	+	+	+
Effects on smooth muscles	++	++	-	-	-	-
Hemolysis indirect	-	-	-	-	-	++
Histamino release	-	++	?	++	-	+
Thromboplasmin Inactivation	-	+	?	?	-	++
Interruption electron Transport	-	+	?	?	-	++
Interruption of phosphorylation oxidative	-	+	?	?	-	++
Effects of distribution	-	-	-	-	++	-

Abstract

Utilizing Apis mellifera Venom as a Biological Control Agent against Vespid parasites (*Vespula germanica*)

The invasive species *Vespula germanica* commonly known as the German wasp poses a significant threat to ecosystem balance and human activities. Traditional control methods often rely on chemical insecticides, which have adverse effects on the environment and human health. As an alternative approach, this manuscript explores the potential utilization of bee venom as a biological agent for controlling *Vespula germanica* populations.

The primary objective of this study is to assess the lethal properties of bee venom in relation to Vespula germanica populations. Through laboratory experiments, the research aims to demonstrate the efficacy of bee venom as a mortality-inducing agent.

The natural venom of honeybees, *Apis mellifera* was applied for the control of *Vespula germanica* adults. Three dose levels of 24, 36 and 56 µl/insect of bee venom were tested against the adult stage of *Vespula germanica*. The adult mortality increased gradually and this parameter correlated with an increase in the doses of bee venom. The LD50 value of the venom was 36 µl/insect after 5 h of adult treatment.

The outcomes of this comprehensive research endeavor will highlight the unparalleled efficiency of bee venom as a biological agent for inducing mortality in *Vespula germanica.*

Keywords: Natural toxins, bee venom, insect control, *Vespula germanica,* German wasp.

Résumé

Utilisation du venin d'Apis mellifera comme agent de lutte biologique contre les parasites vespidés (Vespula germanica).

L'espèce invasive Vespula germanica, communément appelée guêpe allemande, constitue une menace importante pour l'équilibre des écosystèmes et les activités humaines. Les méthodes traditionnelles de lutte reposent souvent sur l'utilisation d'insecticides chimiques, qui ont des effets néfastes sur l'environnement et la santé humaine. Comme approche alternative, ce manuscrit explore l'utilisation potentielle du venin d'abeille comme agent biologique pour contrôler les populations de Vespula germanica.

L'objectif principal de cette étude est d'évaluer les propriétés létales du venin d'abeille par rapport aux populations de Vespula germanica. Grâce à des expériences en laboratoire, la recherche vise à démontrer l'efficacité du venin d'abeille en tant qu'agent provoquant la mortalité.

Le venin naturel des abeilles, Apis mellifera, a été utilisé pour lutter contre les adultes de Vespula germanica. Trois doses de 24, 36 et 56 µl/insecte de venin d'abeille ont été testées contre le stade adulte de Vespula germanica. La mortalité des adultes a augmenté progressivement et ce paramètre était en corrélation avec l'augmentation des doses de venin d'abeille. La DL50 du venin était de 36 µl/insecte après 5 heures de traitement des adultes.

Les résultats de cette recherche globale mettront en évidence l'efficacité inégalée du venin d'abeille en tant qu'agent biologique pour induire la mortalité chez Vespula germanica.

Mots-clés : Toxines naturelles, venin d'abeille, lutte contre les insectes, Vespula germanica, guêpe allemande.

الملخص

استخدام سم النحل (Apics melifira) كعامل مراقبة بيولوجي ضد الطفيليات (Vespula germanica)

ويشكل الأنواع الغازية التي تُعرف باسم الدبور الألماني عموماً تهديداً كبيراً لتوازن النظام الإيكولوجي والأنشطة البشرية. وتعتمد أساليب المراقبة التقليدية في كثير من الأحيان على المبيدات الحشرية الكيميائية، التي لها آثار ضارة على البيئة وصحة الإنسان. وكنهج بديل، تستكشف هذه المخطوطة إمكانية استخدام سم النحل كعامل بيولوجي للسيطرة على مجموعات مبيدات الحشرات.

والهدف الرئيسي من هذه الدراسة هو تقييم الخصائص الفتاكة لسُم النحل فيما يتعلق بسكان *Vespula germanica*. وتهدف البحوث، من خلال التجارب المختبرية، إلى إثبات فعالية سم النحل بوصفه عاملاً يؤدي إلى الوفاة.

طُبقت السم الطبيعي لنحل العسل *Apis melelifera* للسيطرة على البالغين *Vespula germanica* وقد اختبرت ثلاثة مستويات الجرعات من 24 و36 و56 ميكرولتر/حشرة من سم النحل في مرحلة النضج في *Vespula germanica*. وارتفع معدل وفيات البالغين تدريجياً وارتبط هذا المعيار بزيادة في الجرعات من سم النحل. وكانت قيمة الجرعة المميتة المتوسطة للسم 36 ميكرولتر/حشرة بعد 5 ساعات من العلاج.

وسوف تسلط نتائج هذا المسعى البحثي الشامل الضوء على الكفاءة التي لا مثيل لها لسم النحل بوصفه عاملاً بيولوجياً في توليد الوفيات في *Vespula germanica*.

الكلمات الرئيسية: السموم الطبيعية، سم النحل، مكافحة الحشرات، *Vespula germanica*، الدبور الألماني.

Academic year: 2022-2023	**Presented by:** Boulkroune Mohamed Habib Errahmene

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The invasive species *Vespula germanica* commonly known as the German wasp poses a significant threat to ecosystem balance and human activities. Traditional control methods often rely on chemical insecticides, which have adverse effects on the environment and human health. As an alternative approach, this manuscript explores the potential utilization of bee venom as a biological agent for controlling *Vespula germanica* populations.

The primary objective of this study is to assess the lethal properties of bee venom in relation to Vespula germanica populations. Through laboratory experiments, the research aims to demonstrate the efficacy of bee venom as a mortality-inducing agent.

The natural venom of honeybees, *Apis mellifera* was applied for the control of *Vespula germanica* adults. Three dose levels of 24, 36 and 56 µl/insect of bee venom were tested against the adult stage of *Vespula germanica*. The adult mortality increased gradually and this parameter correlated with an increase in the doses of bee venom. The LD50 value of the venom was 36 µl/insect after 5 h of adult treatment.

The outcomes of this comprehensive research endeavor will highlight the unparalleled efficiency of bee venom as a biological agent for inducing mortality in *Vespula germanica.*

Thesis for the Master's degree in Insect Biology and Population Control

Keywords: Natural toxins, bee venom, insect control, *Vespula germanica,* German wasp.

Research laboratory: **Biosystematics and ecology of arthropods.**

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