

**Prospects for new botanical insecticides :
the example of Mediterranean aromatic plants**

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The search for new IPM is the result of half-century of intensive use of synthesized pesticides. During this period, ecological hazards increased alarmingly and counterbalanced the first spectacular successes of organochlorinated pesticides in Italy and North Africa during the second world war. This situation led to a reflection to diversify the approaches for the control of the harmful insects. One of the alternative strategies is the study of the biological activities of the plant defense molecules. These compounds seem to present interest for a more friendly environmental pest management.

1. Plants : a resource of molecules with high potential for pest control

The search for effective control against the phytophagous insects and phytopathogenes are lost "in the mists of time". The search for food constitutes an essential element for the life of all species. With the advent of agriculture, protecting the cultures and preserving harvests become one of major concerns for Human beings.

Phytophagous plants and insects follow a parallel but narrowly interdependent co-evolution: the pollinating insects support the reproduction of the higher plants and the existence of phytophagous insects is subordinated to the presence of vegetable species which constitute their source of food. The study of relations between individuals, populations and species highlights the existence of semiochemical compounds involved in the communication between biological organisms. Among them, plant allelochemicals which take part in the defense of the plant by exerting on the insects a large variety of effects: toxic , repellent , antifeedant etc. These molecules constitute a reserve of new insecticides which can be developed for diversifying IPM strategies.

Compared to the organic synthesized pesticides, the new botanical insecticides have several ecological advantages related to their inherent nature:

- a) as molecules resulting from the secondary metabolism, they have a specificity of action. This fact causes limited attacks of the non targeted organisms. Consequently, they have a positive ecological impact by preserving auxiliary insects and limiting biomagnification .
- b) because of this specificity, they act with low dose on the targeted organism.
- c) as biosynthesized, they are biodegradable with often rather short half-lives. Consequently, they are not very persistent, which represents an appreciable environmental advantage for the balance of the ecosystems.
- d) belonging to various chemical families (mainly polyphenols, terpenes, alkaloids), they proceed from different mechanisms of action which limit the cases of insect resistance (non continuous treatments).

The use of these compounds thus represents a strategy particularly adapted to the actual concerns in regard with biodiversity, absence of toxic residues and a better respect of biocenoses. Moreover, the search for synergist associations, which exist *de facto* in nature, ought to reduce the effective amounts of insecticides. It is well-known since Paracelse, an alchemist of the XVI^e century, that "sola quantitas venenum fecit": it is the amount which makes the poison.

2. Experimental steps for the identification of new botanical insecticidal molecules

Currently, a basic investigation is first to realize an ethnobotanic inquiry to observe the traditional practices to protect crops in the fields or stored products (for example: associated cultures in order to limit the devastations in the field, or plants used in the stockrooms to preserve the stored food)

An ethnobotanic investigation, to be exploitable, must precise the following and essential details for the comprehension of the conditions of efficiency of the plants:

- a) The state of the plant: aspect , odor, maturity at the time of harvest, conditions under which it developed.
- b) The plants to be protected and insects to be targeted. The experiment shows that the results obtained with a species are not always reproducible with another one.
- c) The conditions under which this protection is carried out: open or closed space, temperature, hygroscoy, photoperiod etc...
- d) Finally the type of activity carried on by the plant: insecticidal effect (lethal effect), knock- down effect, repulsive effect etc...

The following stage in the search for new insecticidal molecules is to check in laboratory if these empirical practices have a real basis. The plant to be tested is put in the presence of the insect and one observes the effects of the plant on animal's vitality, reproduction, or behaviour for a period of time. The experiments are carried out according to classical protocols checking the scientific criteria of

relevance and reliability of the results as well as reproducibility. These biotests are accompanied by chemical analyses, especially chromatographic analyses, in order to identify the components of the fractions. However, the phytochemical pattern of the plants is not constant. It is affected by several factors, independently of the variability resulting from the techniques of extractions (pH, solvents, temperature etc) : climate, soil, degree of maturity of the plant, intensity of its metabolism, chemotypes etc. At this experimental stage there is a to and from between the Biology and Chemistry laboratories. The identification of compounds within the extracts leads to new biotests.

Most of the time, there are several compounds in one extract. At the end of this step, molecular associations can be carried out to see if there are synergies between compounds. It is also necessary to determine the range of activity of these compounds by testing them on other species. Then, studies of action mechanisms can usefully supplement this new knowledge: they are of a major interest for the researcher but are not always judged to be useful by the experts of industry in this phase of development of the product. For them, once known the active ingredients, their effectiveness and the insect-targets, it is necessary to pass from the laboratory at the stage R&D of industry: tests in operating conditions, toxicological and ecotoxicological files, choice of a marketing strategy, profitability of the targeted market etc.: a long way in fact for the new biopesticide compound before commercialization !

3. Study of the Mediterranean aromatic plants.

As a consequence of this procedure, the starting point of our research was the observation of ancestral practices to protect kidney bean seeds against the beetle *Acanthocelides obtectus* Say (Bruchidae, Coleoptera). It is a very cosmopolitan insect, which infests not only its plant-host the kidney bean (*Phaseolus vulgaris* L.), but also other Leguminosae like *Cicer arietinum* , *Vigna unguiculata*, *Vicia faba* etc. To prevent infestation of the beetle, farmers of the South-west of France use to put odorous plants like mint, garlic or the bay-tree in the bags of seeds.

So , we tested the efficiency of Mediterranean aromatic plants on the beetle . Plants belonging to the following families, Myrtaceae, Graminae, Umbelliferae, Lauraceae, and Myristicaceae, exerted a protective effect on kidney bean seeds. They acted by causing the death of the insect and by inhibiting the reproduction of the insect. But the most active plants belonged to Lamiaceae : Thyme and wild Thyme (*Thymus vulgaris* and *T.serpyllum*), Rosemary (*Rosmarinus officinalis*), Origan (*Origanum vulgare*).

Let us notice that the mint (*Mentha piperata*) and the bay-tree (*Laurus nobilis*) used by the peasants of South-west developed an action more marked on the reproduction of the beetle. This result shows that the farmers empirically selected plants slowing down the reproduction of the insect in the bags of seeds (that was their purpose).

3.1. Identification of insecticidal compounds

Lamiaceae are famous for their high content in essential oils. The following stage consisted in carrying out a hydrodistillation to extract them. Toxicities of essential oils and botanical residues (after hydrodistillation) are tested on the beetle. Without essential oils, some of these hydrodistilled residues were always toxic to the beetle: Thyme, wild Thyme, Rosemary, Savory (*Satureja hortensis*) and Origan. The insecticidal properties are not the fact of a mere compound in these plants.

3.2. Activity of essential oils and monoterpenes

The insecticidal properties of essential oils on *A. obtectus* were many : fumigant toxicity on the adult insects, inhibition of reproduction by inhibiting oviposition, ovicidal and larvicidal effects as well as antifeedant activity.

The fumigant toxicity was not the same for all the essential oils (table 1). Male insects were more sensitive than female insects. The reproduction of the insect was also affected at various levels: some essential oils acted at the first steps of the reproduction cycle, others at the ultimate steps (table 1). The lethal effect and the inhibition of the reproduction were not always superimposed . For example, Thyme, wild Thyme and Rosemary were very active in both cases, but the Parsley essential oil (*Petroselinum sativum*), which was slightly toxic for the adults, inhibited the reproduction completely. Fumigant toxicity and antifeedant effect which were very marked for the majority of essential oils, presented also differences for some of them. The nutmeg (*Myristica fragans*) essential oil, which has a poor fumigant toxicity on the adults, also decreased the reproduction but not completely. But its antifeedant effect was powerful since no emergence was noted with this essential oils. It appears in this case that the essential oil was briefly inhaled between the moment of hatching and the penetration of larvae inside the seeds while the incorporation of essential oils in artificial seeds provoked a more prolonged contact. Consequently, these properties can be exploitable in two different applications: room fumigation on one side, and impregnation of a wide range of materials on the other.

However the use of essential oils in IPM is delicate because of the multiplicity of their phytochemical patterns. These variations represent a disadvantage for the reliability of plant treatments. To avoid this difficulty, the main compounds of the extracts and their efficient amounts have to be determined for future insecticidal formulations. Therefore, we followed our work by GC chromatography to identify the main compounds of these essential oils. Monoterpenes were the most abundant and they develop an acute toxicity on the adult, as well as activities ovicidal and larvicidal activities at different steps of the reproduction (table 2).

3.3. Activity of polyphenols

The chromatographic analysis of the botanical hydrodistilled residues showed that polyphenolic acids and flavonoides were identified. Rosmarinic acid and luteoline 7-glucoside were the most abundant. They disturbed the natural motility of the insect since the first days, and after eight days all the insects were nearly dead (figure 1). All polyphenols did not acted with the same delay. Some compounds acted quickly: quercetin on the first day, naringine, syringaldéhyde or acid vanillic on the fourth day. Caffeic and ferulic acids, vanillin, lutéoline 7-glucoside induced a knock-down effect. Toxicities of polyphenols were correlated with their attractive effects (Regnault-Roger *et al.*, 2002)

3.4. Sensitivity of the species to the compounds

The activities of the compounds were tested on other species of insects, like *Rhyzoperta dominica* (lesser grain borer), *Ceratitis capitata* (Mediterranean fruit fly); *Rhopalosiphum padi* and *Metopolophium dirrhodum* (corn leaf aphid) for carrying out comparative tests in order to determine their specificities .

It was noted that the most active compounds for one species were not the most toxic for another. For example, linalool which was very toxic on *A.obtectus* was among the less toxic on *C.capitata*. In the same way, anethole was not very active on *A.obtectus* but was very toxic for the others. Thus, it appears that the species developed various sensitivities to the same molecule and that this sensitivity was related to the presence of characteristic chemical structures. *A.obtectus* appeared to be more sensitive to phenolic compounds, *Rhopalosiphum padi* to methoxylated structures although *C.capitata* and *M. dirrhodum* both reacted with no difference to two functional groups . It comes out from this observation that it is necessary to determine for each species which are the most active molecules in a family of insecticidal compounds in order to develop efficient formulations avoiding overdoses.

4. Conclusion

The Mediterranean aromatic plants have insecticidal properties and act both on the adult population and the reproduction at various levels because they contain a wide range of insecticidal allelochemicals

The Mediterranean area is the cradle of Western civilization and offers a profusion of aromatic plants. Its climate is rich in luminosity and heat and with marked seasons as well. This requires adaptive efforts of the plants. This situation is favorable to an evolutionary molecular richness which has conferred multiple properties to aromatic plants. As a consequence, Men employed them very early for diversified uses. Egyptian papyrus mentioned them like condiments, spices or plants medicinal. They were also used in ointments for momies. Today, they are used in many industrial fields:

perfumery, cosmetology, fine chemistry, detergency, pharmacy and food industry. Beside these traditional sectors in full evolution, a new industrial development could be considered: pest control and friendly environmental IPM

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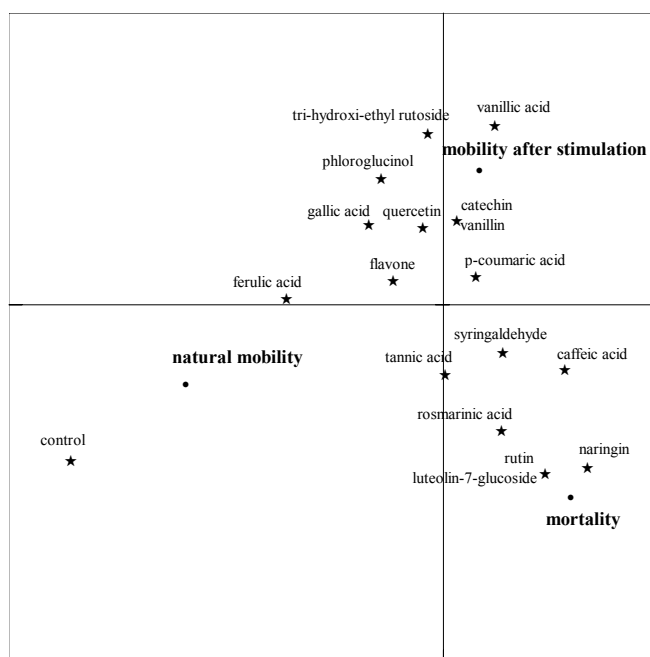
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Day 6



Day 8

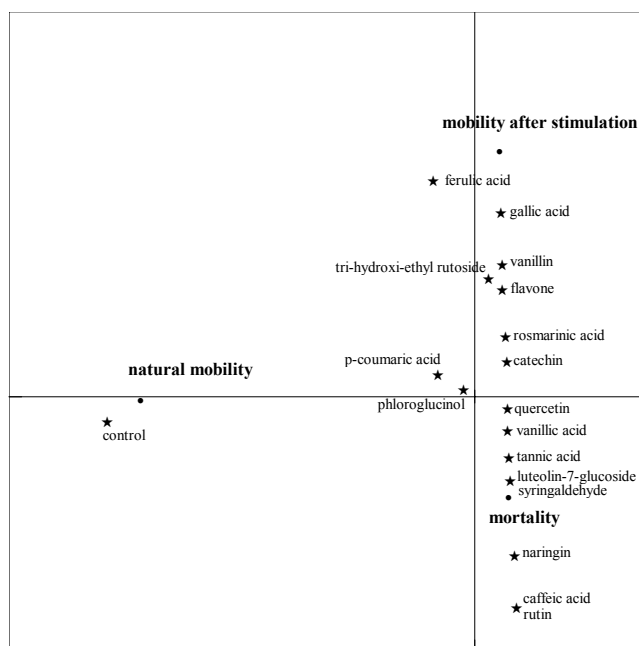


Figure 1: F1x2 factorial map of Correspondence Analysis on the effects of the polyphenolics on mortality and mobility of *Acanthocephalus obtectus* after 6 and 8 days of experiment. Polyphenolic compounds : caffeic acid, ferulic acid, flavone, catechin, quercetin, vanillin, rutin, gallic acid, vanillic acid, phloroglucinol, tri hydroxy ethyl rutoside, tannic acid, p-coumaric acid, syringaldehyde, naringin, luteolin-7-glucoside, rosmarinic acid, control.

Table 1 : Toxicity of essentials oils on *Acanthoscelides obtectus*
(Regnault-Roger et al., 1993 ; Regnault-Roger and Hamraoui, 1994)

Fumigant toxicity		Inhibition of reproduction	
LC ₅₀	Essential oils	Inhibition	Essential oils
LC50 < 10mg/dm³	<i>Thymus serpyllum</i> <i>Thymus vulgaris</i> <i>Origanum majorana</i> <i>Origanum vulgare</i> <i>Cinnamorum verum</i> <i>Rosmarinus officinali</i> , <i>Ocimum basilicum</i> <i>Salvia officinalis</i> <i>Satureja hortensis</i> <i>Coriandrum sativum</i> <i>Cumimum cyminum</i>	Oviposition	<i>Eucalyptus globulus</i> <i>Salvia officinalis</i> <i>Apium graveolens</i> <i>Verbena officinalis</i>
10mg/dm³ < LC50 < 100mg/dm³	<i>Laurus nobilis</i> <i>Verbena officinalis</i> <i>Mentha piperata</i> <i>Anethum graveolens</i> <i>Eucalyptus globulus</i> <i>Citrus limon</i>	larval penetration	<i>Thymus vulgaris</i> <i>Origanum vulgare</i> <i>Eucalyptus globulus</i> <i>Laurus nobilis</i>
LC50 > 100mg/dm³	<i>Myristica fragan</i> <i>Petroselinum sativum</i> <i>Apium graveolens</i>	Emergence Total inhibition	<i>Origanum vulgare</i> <i>Eucalyptus globulus</i> <i>Laurus nobilis</i> <i>Salvia officinalis</i> <i>Coriandrum sativum</i> <i>Thymus serpyllum</i> <i>Thymus vulgaris</i> , <i>Cinnamorum verum</i> <i>Rosmarinus officinalis</i> , <i>Ocimum basilicum</i> , <i>Petroselinum sativum</i>

Table 2 . Toxicity of monoterpenes on *Acanthoscelides obtectus*
(Regnault-Roger and Hamraoui, 1995)

Fumigant toxicity		Inhibition of reproduction	
Toxicity	compounds	Steps of reproduction	compounds
most toxic	carvacrol, linalool, eugenol, thymol,	Oviposition	linalool, thymol, carvacrol
intermediate	Cuminaldehyde, p-cymene, anethol cinnamaldehyde	larval penetration	linalool ,thymol, eugenol, anethole
less toxic	estragol, borneol □-pinene	emergence	carvacrol, linalool, eugenol, thymol, terpineol

