Bioactive compounds in aquatic invasive macrophytes: Exploring the benefits for sustainable agriculture

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Abstract

A lot of fungi in vineyards are harmful to the plant and the grapes. These parasites cause huge yield loss in the vineyard and can also alter the wine taste, which has a cost for the farmers. To counter these threats, chemical industries have invented many pesticides to stop the fungi progression and sometimes to boost the growth of the grapes. However, the use of these chemicals for a long time and to push the vine to the limit is harmful to the environment, killing the biodiversity and impoverishing the soils, in addition to lowering the quality of the wine.

Organic fungicides, which are highly selective for various types of fungal diseases, are considered a promising group of chemical compounds. These fungicides are characterized by persistence and cumulativeness, which creates an ecological toxicological hazard for the ecosystem. Therefore, at present, studies that identify biofungicides on the ecological state of vineyards and the search for ways to reduce their negative aftereffect are becoming relevant. Pesticides used in perennial plantations, accumulating in the soil, reduce its biological potential, migrate into plants, fruits and berries, reducing their quality, biological value and food safety.

In this bibliography review, we defined the different fungi harmful to the vine and their antagonists, in order to discover a compound that will supress pathogens and will be less toxic. So, we are searching for compounds in macrophytes to inhibit fungi growth in the vineyards.

There are several reasons of examining macrophytes as a solution of the mentioned problem. To begin with, macrophytes are known to produce biomolecules (allelochemicals) to inhibit opponent species. The purpose of work is to define which macrophyte extraction may have an adverse effect on the growth of the vineyard's fungi.

During our research we've defined various group of allelochemicals with different properties. Groups of interest for us are triterpenes, flavonoids, coumarins and quinones as they have antifungal properties. So, the suggestion is to find similar compounds in the macrophyte allelochemicals. These allelochemicals were found in such macrophytes as *Typha Latifolia* and *Potamogeton crispus*. Therefore, they are expected to be tested on the vineyard fungi in order to apply or decline the theory.

5 key words: Allelochemicals; Macrophytes; Cyanobacteria; Vineyard fungi; Biocontrol

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We would like to thank Mrs. Juliette Rosebery, our client and sponsor, and Mrs. Nadège Oustrière who supported the project during these three months.

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Introduction

Humanity strives to reduce the environmental impact through many years. Toxic activities in many spheres have already been banned. However, it is still possible to observe the environmental pressure. One of the sources of this pressure is the use of pesticides. Pesticides cause soil contamination, bioaccumulation of toxic compounds and further biomagnification. This fact shows a real threat for the human health and quality of the life.

Here comes biocontrol. The idea of using biomolecules to protect plants was born a long time ago. Nevertheless, biocontrol was not used in a big agricultural farm due to various problems. To start with, biocontrol was far more expensive in comparison with human made compounds and globally less efficient. Biomolecules' extraction requires innovative technologies. Also, the threat of pesticides was not studied enough to define it as a global environmental threat. In this article will be observed the possibility of using macrophyte extracts in a biocontrol way.

The relevance of this topic is incontestable. As far as pesticides were examined, it was found out that it is crucial to search for new measures to protect plants. These measures should not pose any environmental threat, be efficient and available for the consumers.

The goals of the research (Figure 1 : General Schema about the project):

- Find pests in a vineyard
- Examine microphytes' allelochemicals
- Propose a solution for the different vine diseases

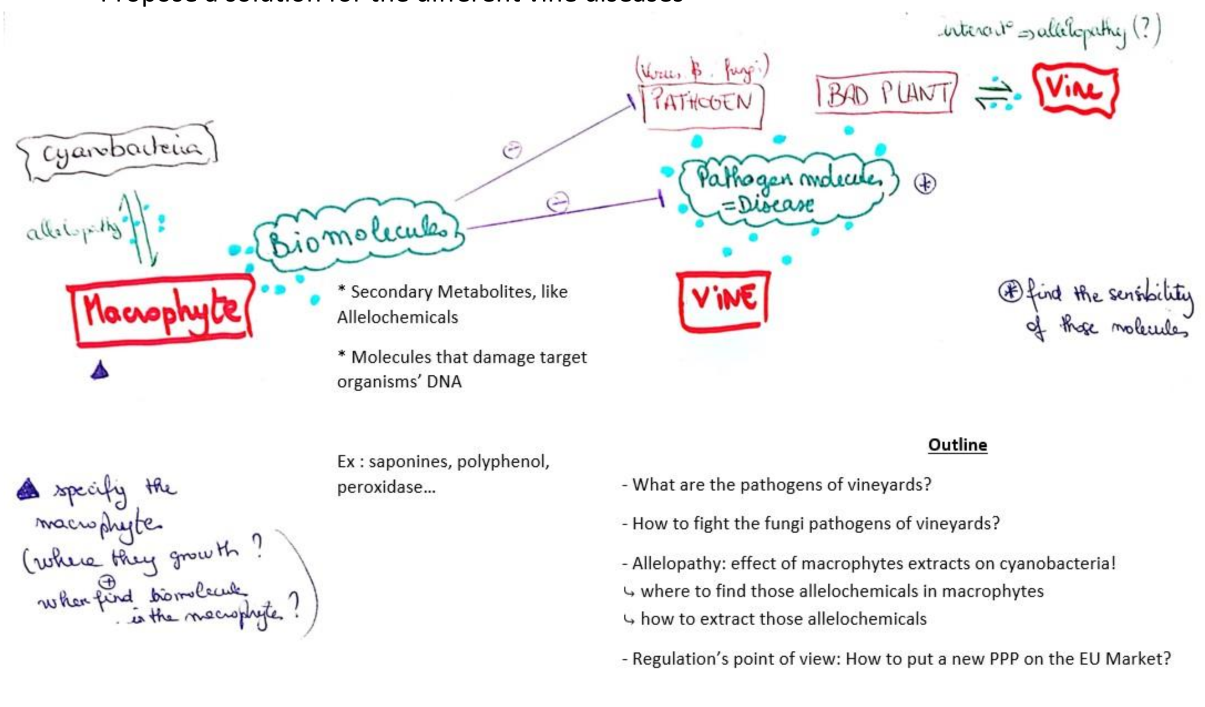


FIGURE 1 : GENERAL SCHEMA ABOUT THE PROJECT

The first chapter of this report will be dedicated to vine diseases. There will be examined different fungi pathogens that cause damage to vineyard yields. The second chapter is about macrophytes and allelochemicals. The third chapter is about regulation and legislation in the sphere of the introduction of a new plant protection product. To sum all up, results will be provided in the conclusion.

1. The pathogen in vine?

The objective of this section is to present the different fungal pathogens of the vine and the diseases they cause. Then, to put forward the molecules which harms the fungi pathogen.

1.1. The fungi pathogens cause disease on vine

1.1.1. Grey rot (botrytis cineria)

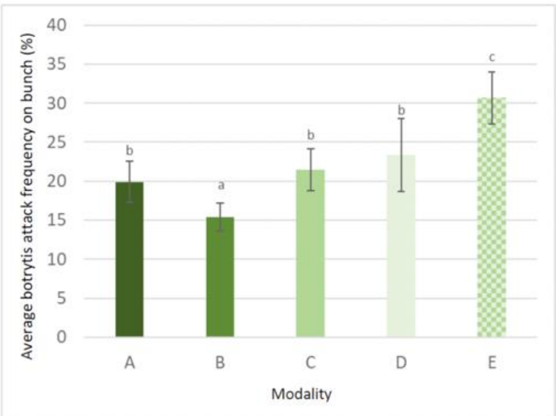
The botrytis or grey mold is a vine disease caused by a fungi called *botrytis cineria.*

He targets all the green parts of the vine, from the branches (with brown spots that turn white) to the leaves (giving a burnt aspect to the shrinking leave) and the grapes (attacking their skin and making them rot with a typical grey aspect).

In winter, the fungi can hide in the fallen leaves or the shoots in the form of scleroses (mycelium gathering). When spring comes with humidity and higher temperatures (between 15-20°C and a 90 % of humidity are the best conditions), the fungi can develop and contaminate nearby tissues with a contamination from close by. In summer, there is an explosive growth of the fungi that can alter most of the harvest and lower considerably the yields.

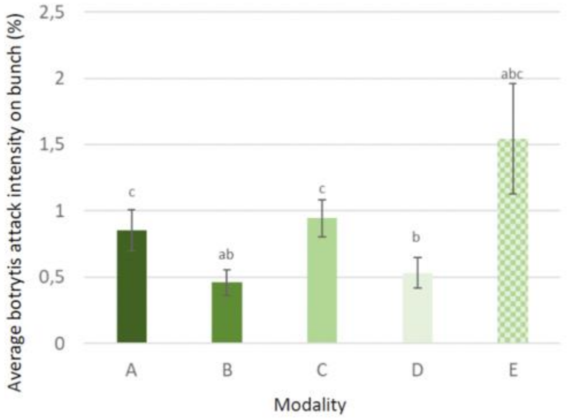
It Is important to know that all the type of vines are not sensitive in the same way to botryitis. When the vine is sensitive and the fungi causes a lot of damages, it is important to treat the plants to prevent the loss of yields.

An efficient biocontrol treatment is the use of potassium bicarbonate. As we can see in the article (Index, 2019) the use of potassium bicarbonate at a rate of two treatments per year (at flowering and at veraison (when the color changes)) significantly lowers the intensity of the attack as we can see on (sFigure 3 : Percentage of average bunch attack intensity on 16 parcels for the different modalities) , where the potassium bicarbonate treatment is the lowest intensity percentage (0,5 %) after the conventional botrytis treatment (0,47 %). Nevertheless, as the (Figure 2 : Percentage of average bunch attack frequency on 16 parcels for the different modalities) graphic shows us, the use of potassium bicarbonate doesn't lower much the frequency of the fungi attacks (potassium bicarbonate is 23 % where the conventional botrytis treatment is 15.5 %).



A = Non treated control,
B = Conventional referenced anti-botrytis (one treatment only during flowering or at bunch closure)
C = foliar fertilizer / CaO+MgO+B (depending on the prescriber recommendations; 4 to 5 applications with a dose of 4kg/ha from the bunch separation phase to the harvest)
D = potassium bicarbonate (2 treatments; at flowering and at veraison (color change)

FIGURE 2 : PERCENTAGE OF AVERAGE BUNCH ATTACK FREQUENCY ON 16 PARCELS FOR THE DIFFERENT MODALITIES



A = Non treated control
B = Conventional referenced anti-botrytis (one treatment only during flowering or at bunch closure)
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D = potassium bicarbonate (2 treatments; at flowering and at veraison (color change)
E = modalities C+D)

FIGURE 3 : PERCENTAGE OF AVERAGE BUNCH ATTACK INTENSITY ON 16 PARCELS FOR THE DIFFERENT MODALITIES

Also, alkali treated saponins in quinoa husks (which is a biproduct of the quinoa production) are useful against the botryitis cineria fungi. As shown in the article (Stuardo & San Martín, 2008) the mycelial growth and conidial germination of the fungi were significantly inhibited. With 5mg of saponins /ml, 100% of conidial germination was inhibited even after 96 hours of incubation. The research proved that the alkali treated saponins created membrane disruption on the fungi's cells where the non-treated saponins had no effect.
This research was conducted in-vitro for the whole process with no tests in real conditions which can be seen as a limit for that experience.
Another article is interesting in the vine defense against *botrytis cineria* is (Jeandet et al., 2002).
This article describes how phytoalexins (more importantly stilbene phytoalexins produced by Vitaceae) can decrease the expansion and inhibit the conidial germination of the fungi on vines. For resveratrol, it ranges from 60 microg/mL (25% inhibition) to 160 microg/mL (100% inhibition)(Adrian et al., 1997). The pterostilbene, a derivative of resveratrol is a more active compounds: It inhibits completely the conidial germination at a concentration ranging from 52 to 60 micro g/ml (Pezet & Pont, 1990).

1.1.2. Downy Mildew (Plasmapora viticola)

The downy mildew is a disease targeting the vines and caused by the fungi *Plasmapora viticola*. This fungus attacks every green and young organs of the plant:
For the young leaves, the symptoms are bleached, yellow and oily spots on the surface, turning into a white fluff. After a while, the contaminated leaves get brown and dry.
The vine shoots are only affected in years of heavy invasion by mildew. During those years, the younger the shoot is, the more vulnerable he will be. When affected by mildew, the vine shoots will not be able to create enough cork to protect themselves in case of frost.

The grapes are receptive to mildew since the beginning of the flowering to the veraison. The symptoms are a change in the grape's color, making them rot.
The fungi are preserved mainly in the form of oospores during winter and becomes mature in early springs. When the temperature gets over 11°C, the oospores sprout, setting free zoospores that contaminate the young vine's organs.

The contamination of the vine by the downy mildew usually impacts the yield up to 30 % which is a huge loss. When the grape gets infected late, it alters the taste and can have a big impact on the wine production for the year.

(Elsharkawy et al., 2014) studied the efficiency of the bacteria Bacillus subtilis, Pseudomonas fluorescens, Derxia gummosa and Trichoderma harzianum to control the powdery mildew and downy mildew diseases on cucumber. This study has been conducted under greenhouse conditions and showed a reduction of the effect of these two mildews on the plant in addition to increasing the volume of the cucumbers compared to the control. The bacteria *T. harzianum* was the most efficient against the downy mildew when AUDPC (area under the disease progress curve) were compared (291.5) but the fungicide Ridomil Gold MZ 68 WG remained in the first place with 241.15 reduction of the AUDPC.

(Feiner et al., 2021) is an article written by Alexander Feiner in 2020 that describes how hops crops acquire downy mildew resistance. After multiple experiments, a small number of metabolites, every one of them being enriched with phenylpropanoids, were found to be correlated with the Downy Mildew Resistance. Their conclusion states that the metabolites enriched with phenylpropanoids are "confirming their protective activity either directly or as precursors of active compounds".
It could be interesting to look for phenylpropanoids enriched metabolites in the vines and if they are linked with the Downy Mildew Resistance.

Chinese researcher Chaoqun Zang and his colleagues wrote an article (Zang et al., 2020) describes an experiment where they tested the antagonistic activity of 303 bacterial strains and *plasmapora viticola* in vitro. 12 strains gave signs of antagonism in a detached leave assay. However, the strain from *Ochrobactrum sp.* stood out in the study with a 93,18 % of disease severity reducing in detached leaves. The bacteria also have antagonistic activity against other vine diseases such as *botrytis cineria*.
It appears that the downy mildew is pretty sensitive to some bacterias and larger research in vivo are needed to see if this disease can be treated with them.

1.1.3. Powdery mildew / Oïdium (erysiph netar or uncinula necator)

The powdery mildew is a fungi disease of the vine, caused by *erysiph necator* or *uncinula necator* and present in almost all French vineyards.
It attacks a lot of the vine parts but has different symptoms:

The young shoots have a slower growth, a shrinking of the leaves and on the most fragile ones a white felting can be observed.
On the leaves, oily spots appear at first and a blackening of the veins follows on the inner side.
The vine grapes that are infected by oïdium get covered by a grey dust then explodes under the pressure of the growing cells. This opening allows other fungi like *botrytis* to enter and infect the grape.
The vine gets less sensitive after the veraison.

The powdery mildew gets through the winter by forming mycelium in the buds (assuring fast contamination of the buds when spring comes) and by stocking "cléistothèces" on the targeted organs (little sacs containing ascospores of the fungi).

It is an external parasite that needs suckers to feed himself from the vine but doesn't need to enter the plant unlike the downy mildew.
This fungus causes a decrease of the yields and alters the wine taste, changing their olfactory profiles.

Cyanobacterias and eukaryote algaes are abundant on earth, simple to grow, and produce a variety of very interesting compounds such as antibacterial and antifungal products. The following article (MM, 1995) written by Martin M. Kulik in 1995 treats about various fungi being inhibited by spraying cyanobacterias solutions during their growth.

In one part of the experiment, turnips infected with *Erysiphe polygoni* and watered weekly with a solution of a commercial seaweed extract (without specification of the seaweed taxon), showed that only 15 % of the total leaf area was infected by powdery mildew, against 85 % for the control experiment.
Another part of the experiment showed that watering strawberry plants with seaweed extract reduced the number of fruits contaminated by *Botrytis cinerea* (gray mold) and increases the fruit yield.
A limit to this test is the growing of in vivo cyanobacterias and seaweeds, to create the same antifungal compounds (it remains to be proven that these substances are produced in nature, Martin M. Kulik (1995)).

It could be useful to test the effect of spraying seaweed on the growth of powdery mildew affecting the vines.

1.1.4. Excoriose (Cryptosporella viticola)

(The excoriose disease targets the vine and is caused by the fungi *Eutypa Lata* and *Cryptosporella viticola*. It is known in France since 1853 under the name of "anthracnose ponctuée" and then arrived in the United States. In English, this disease is called the dead arm.
One of the most destructive diseases of the vine wood. Infection occurs through fresh cuts after pruning. This disease develops slowly. Ulcers at the site of the cut are difficult to detect. It can be noticed that the grapes become infected after 2-4 years. Necrosis occurs in the infected area.
Shoots develop poorly. Infected leaves become small, turn yellow, then wither. The symptoms of the disease become more and more noticeable every year, until seedlings appear on the affected sleeve and the quality of the crop becomes unsuitable.
During winter, the fungi survive in a mycelium form on the shoots. In spring, when the favorable conditions are here (wind or rain to carry the pycnids), the fungi get carried away. When it lands on the vine, either they can penetrate from an already existing breach, or they create an appressorium to enter the vine. There, the fungi will develop, and brown spots will appear.
The major consequence is a deep rot of the trunk and/or the arms of the vine that may cause the death of the organ over the years. That is the reason why it is important to treat the disease as early as possible to prevent it to spread to other shoots.).
The best cure to prevent these fungi is sodium arsenate (Larignon et al., 2009) but it was forbidden in 2001 because of his toxicity on the workers and no other chemical proved its efficiency. The article (Larignon et al., 2009) talks about the potential efficiency of another fungi to compete with *Cryptosporella viticola*. The *trichoderma*'s family, a competitive and hyperparasite fungi, are already used in biological control to fight against diseases and prove to be pretty efficient. They compete against the other fungi and prevent them to grow by overgrowing them and taking their space and resources. Because they aren't harmful to the vine, this competition is positive.

This article also states that "Under practical conditions, no trial has been able to show the effectiveness of such products in protecting pruning wounds against esca or BDA." (Larignon et al., 2009) which tempers the debate and calls for a real experiment under practical conditions.

(Król, 2004) refers to studies that show that the greatest protective activity was detected after using Trichoderma spp., since the number of successfully received infections was significantly lower than in other combinations of the experiment.

The most active in protecting reeds were Trichoderma viride, T.koningii and T. harzianum. The number of successful cases of infection was five, eight and eight, respectively, and these figures were significantly lower than with other methods of treatment (Table 1). The use of Gliocladium spp., bacteria and Euparen 50 WP for the treatment of reeds led to a significant decrease in the number of infected tissues (Table 1 : number of successful infections caused by p.viticola on the canes protected by antagonistic microorganisms (mean for three strains of p. Viticola)).

TABLE 1 : NUMBER OF SUCCESSFUL INFECTIONS CAUSED BY P.VITICOLA ON THE CANES PROTECTED BY ANTAGONISTIC MICROORGANISMS (MEAN FOR THREE STRAINS OF P. VITICOLA)

Microorganism used to canes protection	Number of successful infections				Total	
	'Agat Donskij'	'Iza'	'Prim'	'RF-16'	number	%
Trichoderma harzianum	1	2	2	3	8 a	6.7
Trichoderma koningii	1	1	2	4	8 a	6.7
Trichoderma viride	0	1	1	3	5 a	4.2
Gliocladium catenulatum	4	7	7	8	26 b	21.7
Gliocladium fimbriatum	9	12	11	17	49 d	40.8
Bacillus sp.	14	15	9	18	56 e	46.7
Pseudomonas fluorescens	10	11	12	15	48 d	40.0
Euparen 50 WP	8	9	12	13	42 c	35.0
Positive control	23	26	26	27	102 f	85.0
Total						
number	70	84	82	108		
%	25.9	31.1	30.3	40		

According to the table, it can be noted that the most effective was Trichoderma spp, in comparison with other experiments. Much fewer infections have been reported with Trichoderma spp.

1.1.5. Black rot (Guignardia bidwellii)

The black rot is a cryptogamic vine disease caused by the fungi *Guignardia bidwellii*. It has its origins in the east of North America where it striked the wild vines before the arrival of the European farmed vine. It landed in France in the late 19th century and is today present mainly in the south-west, the west and in Savoy, regions that are favorable for its development.
When the vine is suffering from black rot, the leaves get covered in spots with "dead leave" color and black spots in the periphery. The fruits get attacked later. Round and livid spots appear rapidly at the half-veraison, then turn brown and swallow. In the end, the fruit is totally momified, dryed, and covered in black spots called picnidies.
During winter, the fungi survive on the dryed fruits that weren't picked up or on infected fallen leaves. When spring comes back, the fungi wait for a rain and then projects many spores in the air that get carried away by wind and land on the vine.

Depending on the severity of the attack, the black rot disease can cause a complete crop loss and alter badly the taste of the wine.

According to (Molitor & Beyer, 2014) Spores of *Guignardia bidwellii* germinate poorly on hydrophilic surfaces. These researchers tried using an extract of *Yuca sp.* containing saponins to spray it on the leaves, which had good results in a greenhouse. Unfortunately, any hard weather such as rain would wash off the extract of *Yuca sp.* and leave the vine without defenses after even small amount of rainfall. After seing this, the researchers turned their expectations toward other saponin-containing plants such as *Sapindus mukorossi, Chenopodium quinoa*, *Quilaja sp.* or even extracts from primula roots ((Molitor & Beyer, 2014); (Koch et al., 2013)) but these plants also turned out to be very efficient in greenhouses experiment and not efficient enough when tried on the field.
It could be interesting to find a molecule that fixes the saponin extract on the vine parts and stops it from being washed away by the rain.

(*Phyllosticta Ampelicida)*, 2005) studied the evolution of the vine leaves *in vitro* when treated with different kind of products (all authorized in organic agriculture) and inoculated with the black rot fungi. It emerges that the most efficient of the trial is a product called "Champion" (copper hydroxide) with lime added. We can also witness some activities against black rot leaf infection "by GC-3 (a combination of cottonseed and corn oil and garlic extract), Serenade (Bacillus subtilis) and Armicarb O (organic formulation of potassium bicarbonate)."
After this trial, the team proceeded in another one on the field, placing "mummified grapes" every now and then in the grapevine and on the soil under the grapevine, and treating the vine with the same organic products. The results showed that Amicarb-O, a potassium bicarbonate product close to Milstop, performed rather well with 80 % of the fruits protected while Champion + lime protected only 40 % of the fruits against black rot in limited preliminary cluster innoculation.

1.1.6. Esca (One of the types Grapevine trunk disease)

Esca is a grape disease of mature grapevines. It is a type of grapevine trunk disease. The fungi *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora* and *Fomitiporia mediterranea*.

With this disease, the vine loses its productivity, the yield decreases, the quality deteriorates, which affects the taste components and alcohol content. (Mugnai et al., 1999 ; (Raman & Muthukathan, 2015)).

Sodium arsenate, a pesticide that is effective in killing GEDs and also in reducing the symptoms of Esca disease (leaf striping) (Bonnet, 1926; Rui and Battel, 1963; Swamp and Tosatti, 1977; Del Rivero and Garcia-Marie, 1984), The fungicides benomil and carbendazim have been used to prevent infections caused by eutypiosis and botryosphari. (Magarey and Carter, 1986; Ramsdell, 1995).). However, in the early 2000s, these pesticides were banned in European countries, as they had a negative impact on the environment.

With this disease, the leaves are covered with small colorless spots, which later connect with each other. Only the veins of the leaves do not change color. When white grape varieties are affected, the spots may have a yellow-brown or reddish color. After a while, the infected leaves dries up and falls off.

Also, signs of the disease can be found inside the wood and on the shoots. When cutting the stem, many dark spots are visible in the wood. Cracks form on the bark. Infected bushes grow slowly and bear fruit poorly.

These signs appear in the second half of summer. On white grape varieties, Esca is manifested by yellowing of the leaves, followed by their drying and falling off. In dark forms of grapes, red-brown spots appear on the leaves. They can also be found on berries. A few days after the onset of symptoms, the vineyard dies.

In the review «Fomitiporia mediterranea M. Fisch., the historical Esca agent: a comprehensive review on the main grapevine wood rot agent in Europe», the authors claim that most of the old technique, called "curettage" or "trunk surgery", gives good results, since the symptoms of non-root disease decrease even a few years after curetting. Possibly the most effective method against Fmed. To reduce the penetration of pathogens, it is necessary to protect the wounds from vine pruning. ((Escalen and Gubler, 2001); (Eskalen et al., 2007)). There is no information about studies using some pesticides (based on boskalide or pyraclostrobin and biocontrol products (based on certain strains of

Trichoderma spp.) for the treatment of diseases caused by Fmed. However, they are available in some European countries to protect GTDS. These do not include approved or registered products that contain boscalide, pyraclostrobin or Trichoderma spp. Most of the products and molecules for fighting GTD pathogens have been tested in vitro.

The direct antifungal effect against the growth of Fmed was given by the inclusion of resveratrol in the nutrient medium. The growth of mycelium F med in vitro was insignificantly reduced by compounds of copper oxychloride and gluconate.

1.1.7. Late blight

The causative agent of late blight is Phytophthora infestans. Phytophthora infestans has high variability, specific resistance.
Symptoms appear in wet weather in the form of dirty brown spots. After a while, a light gray coating of spores can be seen on the surface of the spot. Phytophthora is extremely contagious, and spots on the leaves appear already on the 3rd-4th day of infection. If the weather is dry, the leaves quickly dry out and shrivel, in wet weather they droop and rot. Phytophthora is also noticeable on the stems: brown spots without clear borders. Slowly covered with a gray coating of spores. Fruits affected by phytophthora are first covered with dark spots, which slowly spread throughout all tissues. Sometimes the spots are depressed, moist.

Currently, the most reliable approach is integrated management using a number of tactics, the use of "resistant" varieties and the use of fungicides.

In sprinkler systems in which late blight is present, many fungicides are used and this allows the continued use of susceptible varieties, provided that this fungicide is effective. Thus, more than 2,000 tons of fungicides were used to destroy this disease (USA, 2001). However, the cost is about $200 per acre. The use of the fungicide metalaxyl/mefenoxam reduced the effect of late blight.

There are two theories for solving this problem. The first solution is a fungicide, which performs an important function in P.infestans, since the mutations associated with it have not developed, since resistance. Since microbes could adapt quickly to toxins, a permanent effective fungicide is improbable. The second solution is to target the most important effector with the R gene, again with the idea that mutations will be fatal or at least harmful, so that mutants either will not survive or will be at a disadvantage for selection.

The article "The current state and prosperity in the field of biological control of potato late blight (Late blight)" showed that there are many bioagents that can act antagonistically or cause plant resistance.

(Table 3 : List of Macrophytes, Solvent, Cyanobacteria and allelochemicals study in Reviews) showed a large inhibitory role of bioproducts in spore germination. Bioproducts really played an inhibitory role at a very low level of dilution. ENVIRepel is a garlic product, it has been widely used to fight insects, and has also demonstrated an antagonistic role against the pathogen. This role was increased when 0.1% detergent was added to the initial dilution. The use of these bioproducts at a critical moment should reduce the infection rate of P.infestans.

TABLE 2 : EFFECTS ON GERMINATION RATES OF PHYTOPHTHORA INFECTANTS BY DIFFERENT BIOPRODUCTS AT DIFFERENT DILUTIONS

Dilution	Pandorra		A9180		ENVIRepel		ENVIRepel+Del		Daconil	
	Spo	Zoo	Spo	Zoo	Spo	Zoo	Spo	Zoo	Spo	Zoo
4	24,5*	0,1*	6,3	0,5	0*	0*	0*	0*	0*	0*
16	26,3*	3,0*	58,8	68,8	37,0	20,8	32,3*	0,1*	0*	0*
64	33,7*	13,3*	62,5	62,5	62,5	72,0	27,5*	0,1*	0,8*	0*
256	84,3	89,5	83,8	83,3	83,3	55,0	50,5*	17,8*	2,5*	0,1*
1024	71,3	75,7	71,5	85,3	85,3	79,8	57,5	60,0	10,0*	0,5*
4096	65,7	71,3	75,0	62,5	62,5	79,3	57,5	61,5	11,0*	0,7*
16384	76,0	87,3	75,3	78,0	78,0	70,3	68,5	62,0	17,0*	1,0*
CK	75,0	80,0								

Nota: Dilution means the times less than normal solution in usage, Spo.: Sporangium, Zoo.: Zoospore. Each data is the mean of four replicates * : significant difference comparing to CK (P<0.05)

Induced resistance is effective in the fight against late blight and is one of the ways of biocontrol of diseases. Its study can develop new solutions for crop protection. Either the development of transgenic plants that contain molecular components of induced resistance, or by using chemicals that produce a mechanism of resistance to diseases. (Cao Ke-qiang, Forrer H. R. 2001)

2. The Allelopathy: interaction between Macrophytes and Cyanobacteria

Allelopathy is a chemical relationship between different living beings, particularly plants. One living organism produces allelochemicals in order to supress or stimulate the growth of other living organisms. This way it is possible to eliminate natural opponents to get more essential elements and increase the population. However, scientists observe this phenomenon in aspect of the crop protection. The usage of allelochemicals in crop protection may provide an opportunity to decrease the human impact on the environment. Therefore, it will be possible to maintain a sustainable agriculture.

As it was mentioned, allelopathy is a process of chemical interactions between living beings. So, allelochemicals are produced during these interactions. Allelochemicals is a big group of compounds. Every allelochemical has its own properties. These properties depend on chemical structure of allelochemical. Different allelochemicals supress different natural opponents. Moreover, they may supress different systems of the natural opponents. Therefore, it is essential to determine a chemical compound of allelochemical to protect specific crops.

2.1. The types of allelochemicals in macrophytes

Phenolic compounds (-OH: hydroxyl function)

To start with, simple phenolic compounds will be examined. This group of allelochemicals is widespread because phenolic compounds are presented in many plants' species. Also, phenolic compounds are easily found with UV detectors. These factors make phenolic compounds the interesting thing to observe and to consider these compounds in crop protection as the herbicide or fungicide.

Phenolic acids, such as gallic, ferulic, vanillic acids, show their impact on the standard target species, for example on lettuce (Zhang JC et al, 2017). Therefore, scientists are considering simple phenolics as a possible herbicide.

However, simple phenolics are the easiest allelochemicals to observe, it is unpromising that the real application is still not found. Nevertheless, it is essential to widen observations in order to see the effect on the pest species.

Flavonoids compounds are also related to phenolic compounds. These allelochemicals have more complex structure. Flavonoids are mainly produced by rice to supress weed crops. Moreover, flavonoids are used to combat with the wild oat infection in the wheat fields (Liu X et al, 2016).

Flavonoids show good properties in a mitigation of weed. Also, flavonoids are considered as a herbicide due to the water solubility which is vital characteristic.

Coumarins are group of allelochemicals with specific properties. This group is not very phytotoxic. However, coumarins are considered as a measure in a treatment of sclerosis, chemotherapy for multidrug-resistant tumours and organ transplants (Kostova I, 2005). The most phytotoxic compound of coumarins are furanocoumarins. Furan ring is presented in the composition of furanocoumarins.

These compounds may be an object of interest in our work due to its antifungal activity and fungi are the most invasive pest in the vineyard. Antifungal properties are closely connected with the O-substitution and a small aliphatic chain (de Araujo RSA et al, 2013).

Quinones are also derived to phenol group as they have very similar structure. However, not all the quinones are phenolic compounds. Quinones have a strong antimalarial, antibacterial and antifungal effect (Sanchez-Calvo JM et al, 2016). Nevertheless, this group require further research to determine exact properties. Again, as it was mentioned in the simple phenolics, the research was mostly conducted against the standard target species which does not represent the real perspective of use these compounds as a pesticide.

Terpenoids

Terpenoids is a big group of molecules. They are interesting due to therapeutic purposes. Terpenoids can be found in plants and lower invertebrates. The modern science strives to find new methods of extraction of terpenoids.

Monoterpenes are the compounds that can be found in flowers and aromatic plants. These compounds are responsible for the scent of the plant by which we can distinguish it. For instance, the pinene has a piney smell and the limonene has a citrus odour. Also, some monoterpenes have an antibacterial and antifungal properties (Aliberti L et al, 2016). However, monoterpenes also interact with different plants and even insects. Moreover, limonene extracts can not only supress, but stimulate the germination and growth. In the research of the American scientists this interaction was shown with the grass *Bouteloua curtipendula* in the slopes of Texas (Young GP et al, 2009). Monoterpene α-pinene attracts *Rhagoletis completa*. The knowledge about this interaction may be used in terms of protection of walnuts and use of the insecticide at the time of the α-pinene emission (Sarles L et al, 2017). Limonene is also used before the pesticide treatment due to its ability to supress the germination of the barnyard grass seed (Gouda NAA et al, 2016).

Antifungal activity of the monoterpenes can be represented by α-phellandrene, o-cymene and β-ocimene. These compounds are extracted from the *Senecio amplexicaulis*. They inhibit fungi and have phytotoxic effect on *Phalaris minor* and *Triticum aestivum* (Singh R et al, 2016).

Sesquiterpenes are group of compounds which play a significant role in the plant defence system. Sesquiterpenes are responsible for a plant-insect, plant-plant and plant-fungi relationship. Compounds have an antifeedant properties in order to protect plants from the animals (Winters AE et al, 2018).

Production of sesquiterpenes is highly correlated with the stress and soil composition as a main source of stress. High concentration of nitrogen and phosphorus lead to the appearance of sesquiterpenes in the leaves of *Pinus halepensis*, *Rosmarinus officinalis* and *Citus albidus* (Wu B et al, 2010).

Perspectives of use sesquiterpenes as an herbicide are being observed by the scientists. It was found out that sesquiterpenes suppress such plants as tomato, onions, lettuce, watercress, barnyard grass and brachiaria (Miranda MAFM et al, 2015).

Diterpenes are the wide-spread compounds. It can be found in fungi, plants, and animals. Organisms produce diterpenes in order to protect them. Also, these compounds are efficient against algae (Rasher DB et al, 2011).

Triterpenes are heavy compounds that consist of C30H48 group. Their chemical structure makes them easily transform into another secondary metabolite. For example, triterpenes often become sterols, steroids and saponins (Kushiro T et al, 2010). This feature gives us an opportunity to create an herbicide due to phytotoxic properties of the transformed compounds. It was found that those compounds have an ability to suppress the photosystem II. The problem is that triterpenes are complex compounds and mostly show low activity which is a big a con in case of use as a pesticide.

Betulinic, oleanolic and ursolic acids supress mainly monocotelydons (Wang CM et al, 2014). However, the problem of use of these acids in biocontrol is their high stability in the soil. They are low water soluble and can persist in the soil for a long time.

Nevertheless, there are triterpenes that are not persistent. For instance, protodioscin is not persistent in the soil and exhibit phytotoxic properties against soybean seedlings. This compound can be found in the *Urochloa ruziziensis* (Nepomuceno M et al, 2017).

Compounds with a nitrogen atom

Alkaloids are biomolecules that contain at least one nitrogen atom. Mostly, alkaloids are produced from the amino acids. However, they can be also produced from triterpenes. Due to the properties of the alkaloids, they are used in the pharmacy. However, in the terms of allelochemicals, the main purpose is to protect the plant from the herbivorous organisms. Alkaloids can be produced in the different cell organelles, and they are mainly stored in the vacuoles due to the water solubility of compounds (Mu HM et al, 2010).

Alkaloids play a significant role in the aqueous ecosystem. Cyanobacteria contain a large amount of nitrogen which leads to production of alkaloids. Therefore, cyanobacteria inhibit the growth of the coral reproduction (Ritson-Williams R et al, 2016). So, cyanobacteria's allelochemical suppress the macrophytes and vice versa. Macrophytes also produce alkaloids in order to inhibit the photosystem II which is essential for the cyanobacteria.

Alkaloids have different modes of action. As it was mentioned, alkaloids may inhibit the photosystem II. However, it is more common to observe the DNA, RNA and enzyme suppression (Filippin KJ et al, 2018). Alkaloids may also interact with the auxin receptor. Auxin is an enzyme responsible for the plant growth. Therefore, alkaloids affect the plant growth (Ortiz-Castro R, 2011).

Benzoxazinoids are of interest in biocontrol research. These nitrogen containing compounds show a strong effect on weed such as *Eleusine indica, Sonchus avensis, Lamium amplexicaule, Alopecurus aequalis, Kummerowia striata, Rumex japonicas, Chenopodium serotinum, Taraxacum mongolicum and Capsella bursa-pastoris* (Zhang S-Z et al, 2016). These weeds are present in the field and mitigation with them require the use of herbicides. In order to reduce the bad impact on the environment, it is possible to use benzoxazinoids.

Also, one of the important factors is benzoxazinoids are present in a huge quantity in widespread plants, for instance they are presented in the rye (Cimmino A et al, 2015). Therefore, we will not face a problem of producing benzoxazinoids.

Nevertheless, some pests are resistant to the benzoxazinoids. N-glucosylation detoxicates benzoxazinoids and presents in the fungi. This way, benzoxazinoids are not efficient against the fungi contamination (Schulz M et al, 2016).

2.2. The effect of those allelochemicals on cyanobacteria

For environmental reasons, we seek to reduce the number of herbicides, and pesticides more generally. Thanks to allelochemicals, and to the models of interactions by allelopathy between macrophytes and cyanobacteria, we think, as (De Albuquerque et al., 2011) that we could replace artificial herbicides manufactured by man by natural algaecides provided by allelopathy. Thus, the extraction, separation, identification, and synthesis of allelochemicals is an important subject of study.

We have considered multiple reviews, in which macrophytes, cyanobacteria, allelochemical biomolecules and extraction solvents are involved.

The list follows (Table 3 : List of Macrophytes, Solvent, Cyanobacteria and allelochemicals study in Reviews):

TABLE 3 : LIST OF MACROPHYTES, SOLVENT, CYANOBACTERIA AND ALLELOCHEMICALS STUDY IN REVIEWS

Macrophytes:

- *P. Crispus*
- *M. Spicatum*
- *N. Marina*
- *C.Demersum*
- *P. Pectinatus*
- *R. aquatilis*
- *N. officinal*

Cyanobacteria :

- *M. Aeruginosa*
- *O. Tenuis*
- *P. Subcapitata*
- *Anaebana Sp.*
- *A. variabilis*

Allelochemical families :

- Alkaloids
- Ketones
- Terpenes
- Fatty acids
- Fatty acids alcohol
- Esters
- Hydrocarbons
- Sterols
- Phenolic compounds → polyphenols
    - Tellimagrandin
    - (+)-catechin (CA) (belong to the flavonoids family)
    - pyrogallic acid (PA)
    - ellagic acids (EA)
    - Gallic Acids (GA)
    - Tannic Acid (TA)

Solvent :

Polar		Medium Polar	Low polar
Protic	Aprotic		
Ethanol Methanol	Acetone	chloroform	Methylene chlorid Petroleum ether (lipophilic)

2.2.1. Choice of solvent

In the review (Haroon & Abdel-Aal, 2016), we can read the properties of each solvents used to extracts 66 different types of allelochemicals and anti-algal compounds from two macrophytes (*Potamogeton crispus* and *Myriophyllum spicatum*).

We can see that concentration and number of allelochemicals depend on the plant species and the polarity of solvent.

In fact, for low polar solvent, like petroleum ether, methylene chloride, the alkaloids family are not present in any extracts, contrary to the other families. Petroleum ether permit to extract the highest number of chemical compounds. (Table 5 : numbers of chemical compounds related to each group in the 2 macrophytes different extracts).

For medium polar solvent (like chloroform), the alkaloids, terpenes, and sterols are not present in the extracts from *P. Crispus*. Using Chloroform as solvent provide the lowest number of chemicals compounds. (Table 4: phytochemical constituents of p. crispus and m. spicatum different extracts by gc-ms spectra)

If the polar solvent acetone, all nine major chemical groups (alkaloids, ketones, terpenes, phenolic, fatty acids, fatty acid alcohol, esters, hydrocarbons, and sterols) are present.

The highest percentage of extraction yield was obtained with methanol as solvent but use methanol as solvent don't permit to extract phenolics and hydrocarbons from M. spicatum, or sterols from P. crispus.

Furthermore, Ketones represent the main chemical compounds of *M. spicatum* extracts (27 % of total crude extract), and on the other hand, hydrocarbons and sterols are the two allelochemical present in less amount in both macrophytes extracts. (Figure 4 : Percentage concentration of each identified chemical in p.crispus and m. spicatum extracts)

We can understand that more a solvent is polar, more the yield of allelochemicals extraction will be important.

Globally, the highest inhibition effect of those compounds on phytoplankton (*P. subcapitata*) could mainly be caused by high concentrations of phenolic compounds (in addition to the presence of special phenolic compound (Phenol,3- (1,1-dimethylethyl)-4-methoxy–).

TABLE 4: PHYTOCHEMICAL CONSTITUENTS OF P. CRISPUS AND M. SPICATUM DIFFERENT EXTRACTS BY GC-MS SPECTRA

Compound (IUPAC-name)	Retention time (Min)	Molecular ion peak (*m/z*)	Concentrations, mg/100 g DW											
			Petroleum ether		Methylene chloride		Chloroform		Acetone		Methanol		Sum.	
			P. crispus	*M. spicatum*	*P. crispus*	*M. spicatum*	*P. crispus*	*M. spicatum*	*P. crispus*	*M. spicatum*	*P. crispus*	*M. spicatum*	*P. crispus*	*M. spicatum*
Alkaloids														
2,4-Dimethylpyridine	5.24	107	0	0	0	0	0	0	0	10.373	0	70.538	0	80.911
4-Piperidinone, 2,2,6,6-tetramethyl-	8.59	155	0	0	0	0	0	0	8.694	14.173	273.82	867.84	282.52	882.01
1H-Pyrrole-2,5-dione,3-ethyl-4-methyl-	10.9	139	0	0	0	0	0	0	0	0	254.79	124.73	254.79	124.73
Sum.			0	0	0	0	0	0	8.694	24.546	528.61	1063.11	537.31	1087.65
Ketones														
3-Penten-2-one, 4-methyl-	3.32	98	0	0	0	0	0	0	1.8589	6.84	320.34	482.13	322.2	488.97
2-Pentanone, 4-hydroxy-4-methyl-	3.85	116	0	0	0	0	0	0	8.263	186.92	353.11	870.98	361.38	1057.9
2-Pentadecanone, 6,10,14-trimethyl-	19.9	268	161.24	259.17	125.32	249.05	56.687	9.7146	56.891	53.2	243.16	285.09	643.3	856.23
Sum.			161.24	259.17	125.32	249.05	56.687	9.7146	67.0129	246.96	916.61	1638.2	1326.88	2403.1
Terpenes														
(7aR)-5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	15.8	180	9.084	26.2	0	8.2745	0	0	0	0	57.619	187.61	66.703	222.09
2,6,10,14-Tetramethylpentadecane	18.4	268	4.542	6.7573	0	10.323	0	0	0	0	0	0	4.542	17.08
(−)-Loliolide	19.2	196	0	0	44.585	35.474	0	0	7.6399	0	179.73	78.923	231.95	114.4
Neophytadiene	19.8	278	0	0	269.92	58.827	0	0	60.858	61.286	104.25	27.775	435.03	147.89
2,6,10,14-Tetramethylhexadecane	20.8	282	5.4496	14.702	0	76.109	0	0	0	0	0	0	5.4496	90.811
(2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	23.2	296	21.196	21.016	241	31.787	0	0	103.16	90.789	133.21	82.801	498.57	226.39
4,8,12,16-Tetramethyl-heptadecan-4-olide	25.8	324	13.672	71.249	0	14.255	0	0	0	0	0	43.916	13.672	129.42
Sum.			53.9436	139.924	555.505	235.05	0	0	171.658	152.075	474.809	421.025	1255.92	948.081
Phenolics														
2-Methoxy-4-vinylphenol	12.3	150	3.8863	0	7.23	0	14.457	2.1901	0	0	0	0	25.573	2.1901
Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	14.7	180	0	0	0	0	28.153	1.1381	0	0	0	0	28.153	1.1381
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	15.3	220	0	0	130.14	99.13	324	42.997	4.968	0	0	0	459.11	142.13
Phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	26.6	340	30.824	41.75	72.3	303.37	187.2	87.337	0	0	0	0	290.32	432.46
Sum.			34.7103	41.75	209.67	402.5	553.81	133.662	4.968	0	0	0	803.156	577.918
Fatty acids														
Tetradecanoic acid	18.9	228	6.813	17.341	0	21.219	0	0	0	4.8578	96.525	0	103.34	43.417
Hexadecanoic acid	21.6	256	17.411	0	0	0	0	0	2.5432	12.324	1309.9	133.11	1329.9	145.43
9- Octadecenoic acid, (E)	23.5	282	10.697	20.922	15.665	98.311	13.221	1.3705	7.6399	6.7065	0	20.543	47.222	147.85
Sum.			34.921	38.263	15.665	119.53	13.221	1.3705	10.1831	23.8883	1406.43	153.653	1480.46	336.697
Fatty alcohols														
1-Hexadecanol	19.5	242	0	2.7048	0	0	0	0	0	0	92.719	155.12	92.719	157.83
1-Octadecanol	20.4	270	4.542	4.5436	85.194	0	35.352	0	7.7004	16.484	0	31.108	132.79	52.136
1-Icosanol	24.8	298	0	0	0	21.464	0	0	20.53	10.578	107.84	67.813	128.37	99.856
Sum.			4.542	7.2484	85.194	21.464	35.352	0	28.2304	27.062	200.559	254.041	353.879	309.822

TABLE 5 : NUMBERS OF CHEMICAL COMPOUNDS RELATED TO EACH GROUP IN THE 2 MACROPHYTES DIFFERENT EXTRACTS

Chemical groups	*P. crispus*					*M. spicatum*				
	P	Me	C	A	Mt	P	Me	C	A	Mt
Alkaloids (3)	–	–	–	1	2	–	–	–	2	3
Ketones (3)	1	1	1	3	3	1	1	1	3	3
Terpenes (7)	5	4	–	3	4	5	7	–	2	5
Phenolics (4)	2	3	4	1	–	1	2	4	–	–
Fatty acids (3)	3	1	1	2	2	2	2	1	3	2
Fatty alcohols (3)	1	1	1	2	2	2	1	–	2	3
Esters (12)	5	4	2	6	3	9	4	4	5	6
Hydrocarbons (16)	10	3	2	4	–	14	8	5	–	–
Sterols (6)	4	3	–	1	–	6	3	–	1	2
Others (9)	4	2	1	2	4	6	2	1	2	3
Total No. of compounds (66)	35	22	12	25	20	46	30	16	20	27

The numbers between brackets represents the total numbers of identified compounds related to each chemical group. P = Petroleum ether, Me = Methylene chloride, C = Chloroform, A = Acetone and Mt = Methanol.

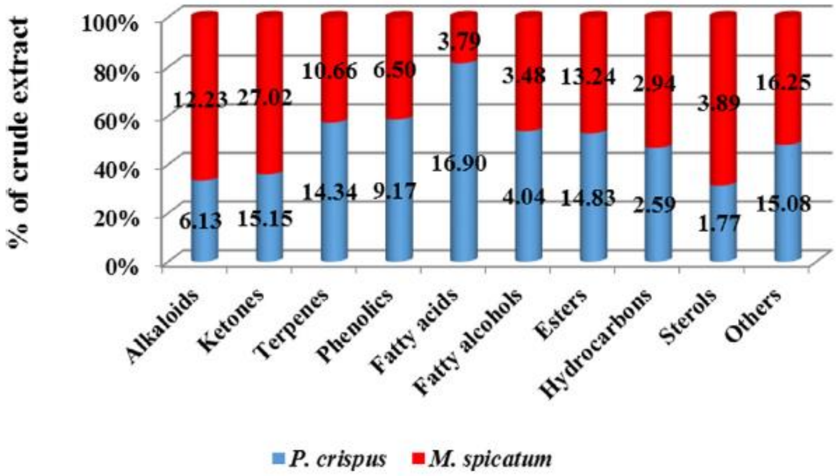

FIGURE 4 : PERCENTAGE CONCENTRATION OF EACH IDENTIFIED CHEMICAL IN P.CRISPUS AND M. SPICATUM EXTRACTS

For *C. demersum*, we can assume the presence of other allelochemicals, but for now, they are still unknown.

According to (Ghobrial et al., 2015) both 50% and 100% aqueous acetone and ethanol solvent from the extracts of the 2 macrophytes (*P. pectinatus* and *Ceratophyllum demersum*), exerted inhibitory activities against the two cyanophytes (*Microcystis Aeruginosa* and *Oscillatoria Tenuis*). In those conditions, using ethanol as an extraction solvent shows that both macrophytes have a high inhibitory effect on the rate growth of the bloom, unlike using Acetone, which is less important inhibitor effect.

2.2.2. Bioactivity of the allelochemicals on cyanobacteria development

(Tazart et al., 2019) said that *R. aquatilis* and *N. officinal* extracts, composed by total phenols (TPs), total flavonoids (TFs), and tannins (TTs), have a concentration-dependent negative impact on *M. aeruginosa* growth. Only after 8 days of treatment, 100% and 75 % repectively of rate of inhibition have recorded. In fact, the Chlorophyll-a and carotenoid concentrations were decreased, colonial and cell and colonial morphology changed and cyanobacteria cells sedimented.

We can affirm that *R. aquatilis* and *N. officina* may control the tested cyanobacteria and may be useful to control harmful blooms in lake-reservoirs.

In general, the allelopathic activity of *N. marina* was weaker than that of *C. demersum* on cyanobacteria (Gross et al., 2003). The strongest activity against the two cyanobacteria *Anabaena sp. PCC 7120* (=SAG 25.82) and *A. variabilis strain P9* (=ATCC 29413) was achieved by extracting plant tissue with 50% methanol + indicating that hydrophilic and lipophilic substances are allelopathically active.

(Chen et al., 2012) have investigate the effects of 8 species (*Zizania aquatica, Typha orientalis, Iris wilsonii, Phragmites australis, Arundo donax, Nymphaea tetragona, Nelumbo nucifera,* and *Alternanthera philoxeroides*) of aquatic macrophytes on the growth of the cyanobacteria M. aeruginosa (*Attention, for this document, we would like to alert you to the fact that despite the title and content of the review, the so-called macrophytes studied do not seem to belong to the macrophyte family* !!). After 19 days incubation, they found that 0.1–1% autoclaved leaves add to 1% extract from fresh leaves of *Iris wilsonii* have significantly inhibited the growth of *M. aeruginosa*. This can be explained by the fact that the autoclave promotes the release of these anti-cyanobacteria molecules.

Besides, although all extracts of the 8 macrophytes can significantly inhibit the cyanobacteria *M. aeruginosa*, some conditions are more or less effective.

For *N. tetragona* and *N. nuciferathe*, the leaves have more inhibitory effect than their petiole, but it is the leaves of *Nymphaea tetragona, Typha orientalis, Nelumbo nucifera* and *I. wilsonii* that show the most potent inhibitory power (75-82% inhibition of cyanobacterial growth after 19 days of exposure).

Thereafter the same team have determined the total phenolics and tannin contents in the macrophytes in order to reveal the nature of cyanobacteria inhibition. They notice *M. aeruginosa* decreases in cell density when exposed to increasing levels of total phenolics and tannins in macrophyte tissue.

Once again, although unknown at the moment, other allelochemical molecules must come into play to inhibit the growth of cyanobacteria. (Christiansen & Fenchel, 1977) have mainly pinpoint those others allelochemicals as being steroids, flavonoids, alkaloids, terpenoids, and organic cyanide.

(Zhou et al., 2019) investigated the effect of allelochemical anti-cyanobacterial molecules released by two plants (*one more time, note that despite the title and content of the journal, the so-called plants studied do not appear to be members of the macrophyte family. However, these two plants can be used as artificial floating beds and grow in water*) *C.alternifolius* and *C.generalis* on the cyanobacterium *M. aeruginosa*. The conclusion remains the same as before: through GC/MS analysis they noticed no less than 15 different families of compounds such as fatty acids and phenolic compounds.

This following table (Table 6 : Summary of inhibition effect of macrophytes on the maximum growth of cyanobacteria represented using the EC50 value [g-wet /l]) shows the EC50 concentrations correlated to the inhibitory effects of different species of macrophytes on algal growth (Nakai et al., 1999).

TABLE 6 : SUMMARY OF INHIBITION EFFECT OF MACROPHYTES ON THE MAXIMUM GROWTH OF CYANOBACTERIA REPRESENTED USING THE EC50 VALUE [G-WET /L]

Macrophy \ Alga	*M. aeruginosa*	*A. flos-aquae*	*P. tenue*
E. densa	7	3	-b
C. caroliniana	8	7	7
M. spicatum	1	3.5	2
C. demersum	2.5	5	-
E. acicularis	2	3	-
P. oxyphyllus	2.5	-	-
P. crispus	-	-	-
L. sessiliflora	5	-	4.5
V. denseserrulata	5.5	-	-

[a]: Data obtained from Nakai *et al.* (1996).
[b]: No inhibition or weak inhibition, *i.e.* impossible to inhibit maximum growth by 50% of control at a macrophyte concentration of 0 to 10 g-wet l^{-1}.

E. densa, C. caroliniana, M. spicatum, C. demersum, and *E. acicularis* shows significant inhibition on the cyanobacteria *A.fios-aquae*.
While the cyanobacteria P. tenue is inhibited by the allelochemicals produced by *C. caroliniana, M. spicatum,* or L*. sessiliflora*.
And *M. aeruginosa* is inhibited by all macrophytes except *P. crispus*.

For next experiments, (Zhou et al., 2019) will choose the species of macrophytes *M. spicatum*, because it have the second strongest growth inhibition of *A. jlos-aquae*, and the strongest effects on both *M. aeruginosa* and *P. tenue*.

2.2.3. Some physiological processes (inhibition) on target cyanobacteria

According to (Wang et al., 2011) the active compound Tellimagrandin II is a polyphenol allelochemical released by *Myriophyllum spicatum* (macrophytes in freshwater). This allelochemical provide a shift of the maximum emission temperature by 12°C.

Tannic and gallic acid, as well as (+)-catechin also caused a comparable, dose-dependent shift of the maximum temperature of the B-band of PSII-enriched membrane fragments (in the photosynthesis metabolism).
Tellimagrandin II is responsible (with certainly a synergistic effect with other polyphenols) for the inhibition of PSII activity.

We can conclude that Tellimagrandin II has at least two modes of action : inhibition of exoenzymes and inhibition of PSII (Wang et al., 2011).
Multiple target sites are a common characteristic of many potent allelochemicals (Wang et al., 2011).

Those other polyphenols produced by *Myriophyllum spicatum* have also one other mode of action that inhibit the cyanobacteria. In fact, (+)-catechin (CA) (=flavonoids, polyphenols) and pyrogallic acid (PA) (= polyphenols) could induce ROS formation in *Microcystis aeruginosa* and *Pseudokirchneriella subcapitata* (2 cyanobacteria) (Wang et al., 2011).

The redox cycling of certain allelochemicals may be one factor for the algicidal activities in natural ecosystems by inducing ROS generation (Wang et al., 2011).

There is biotic and abiotic factor that influence this phenomenon: light can enhance the induced by Catechin ROS formation in the two species of cyanobacteria (*Microcystis aeruginosa* and *Pseudokirchneriella subcapitata*).
But it is not the case if ROS formation is induced by PA (due to the levels of the intracellular O2 and reductant NAD(P)H) (Wang et al., 2011).

(Zhu et al., 2010) and (Leu et al., 2021) reported that PA (released by the macrophyte *Myriophyllum Spicatum*) could reduce photosystem II and whole electron transport chain activities of *M. aeruginosa.*

This can be explaining the reasons why light have no enhancing effect on PA-induced ROS generation, since the production of both intracellular O2 and reductant NAD(P)H, which are necessary for the redox cycling reactions of PA in the test organisms, decreases due to the inhibition of the photosynthesis.

Furthermore, (Zhu et al., 2010) shows that the non-photochemical quenching (NPQ) and effective quantum efficiency (YII) of the cyanobacteria M. aeruginosa were affected earlier and more rapidly than its growth rate when they coexisted with macrophyte *Myriophyllum spicatum*.

In fact, as said previously, the *M. Spicatum* can release allelopathic polyphenols like pyrogallic acid (PA), gallic acid (GA), ellagic acid (EA) and (+)-catechin (CA).

They also evaluated the combined effect of *M. spicatum* extract, composed by four polyphenols. They can assume the presence of synergic effect by them on the NPQ, YII and growth of the cyanobacteria *M. aeruginosa*.

Once again, this teams highlighted pyrogallic acid and gallic acid caused significant reductions of photosystem II (PSII) and whole electron transport chain activities of M. aeruginosa, whereas the dark respiration and photosystem I (PSI) activities were significantly increased. Ellagic Acid had no influence on the electron transport activities of the tested organisms, so for Catechin, contrary to what it was said before.

The inhibition of undesired cyanobacteria by macrophytes can be caused by the synergistic effect of allelochemicals plus the reduction in photosynthetic activity. And for good reason, Photosystem II must be attacked by the allelopathic polyphenols.

The (Table 7 : Summarize of Allelopathy effect of Macrophytes on Cyanobacteria) is a summary table that combine whole information about allepathy that the group found.

TABLE 7 : SUMMARIZE OF ALLELOPATHY EFFECT OF MACROPHYTES ON CYANOBACTERIA

Macrophytes species	Allelochemicals	Family of allelochemicals	Cyanobacteria inhibited
Acorus tatarinowii, Acorus calamus, Acorus gramineus	Phenylpropanes	Phenolic compounds	Anabaena flos-aquae, Aphanizomenon flos-aquae, Microcystis aerugninos Synecococcus leopoliensis
Arundo donax	Gramine	Alkaloids	Microcystis aeruginosa
Brasenia shcreberi			Anabaena flos-aquae
Cabomba caroliniana			Microcystis aeruginosa, Dolichospermum flosaquae (formerly Anabaena flos-aquae), Leptolyngbya tenuis (formerly Phormidium tenue), Cyanobacteria as a whole
Ceratophyllum demersum			Microcystis aeruginosa, Pseudanabaena limnetica (formerly Oscillatoria limnetica), Oscillatoriales. Anabaena sp., Trichormus variabilis (formerly Anabaena variabilis), Aphanizomenon flos-aquae, Synechococcus elongatus,

			Cyanobacteria as a whole
Chara aspra	4-methylthio-1,2-dithiolane and 5-hydroxy-1,2,3-trithiane		Anabaena cylindrica, Anabaena torulosa, Anabaenopsis elenkinii, Microcystis aeruginosa, Microcystis flos-aqua, Synechococcus sp., Cyanobacteria as a whole
Eichhornia crassipes	N-phenyl-1-naphthylamine	Nitrogen containing compounds	Dolichospermum flosaquae (formerly Anabaena flos-aquae), Romeria leopoliensis (formerly Synechococcus leopoliensis), Microcystis aeruginosa, Anabaena azollae
Elodea Canadensis	Phenolic compounds	Simple phenolics	Microcystis aeruginosa, Anabaena spp., epiphytics cyanobactera
Hydrilla verticillata	Phenolic compound (vanillic acid, protocatechuic acid, ferulic acid, caffeic acid)	Simple phenolics	Dactylococcopsis sp., Microcystis aeruginosa
Myriophyllum brasiliense	Polyphenols	Phenolic compounds	Microcystis aeruginosa, Dolichospermum flosaquae (formerly Anabaena flos-aquae)
Myriophyllum verticillatum	A-asarone, phenylpropane, glycoside-like allelochemicals	Phenolic compounds	Limonothrix redeke, Microcystis aeruginosa
Myriophyllum Spicatum		Ketones Alkaloids Esters mostly	Microcystis aeruginosa, Dolichospermum flosaquae (formerly Anabaena flos-aquae), Leptolyngbya tenuis (formerly Phormidium tenue); Cyanobacteria as a whole
N. Marina			Anabaena sp., Trichormus variabilis (formerly Anabaena

			variabilis), Synechococcus elongates, Cyanobacteria as a whole
Nasturtium officinale	flavonoids	phenols, and tannins	Microcystis aeruginosa,
Nelumbo nucifera	Propenamide	Nitrogen containing compounds	Microcystis aeruginosa
Potamogeton crispus	• Alkaloids • Ketones • Terpenes • Fatty acids • Fatty acids alcohol • Esters • Phenolic compounds → polyphenols		Trichormus variabilis (formerly Anabaena variabilis), Cyanobacteria as a whole
Potamogeton pectinatus			Microcystis aeruginosa, Oscillatoria tenuis
Pistia stratiotes	Polyphenols, linolenic acid, fatty acids, steroidal ketones		Synechococcus leopoliensis, Microcystis aeruginosa
Ranunculus aquatilis	flavonoids	phenols, and tannins	Microcystis aeruginosa
Typha angustifolia	Phenolic acids (o-hydroxycinnamic acid, syringic acid and isoferulic acid)	Simple phenolics	Dolichospermum flosaquae (formerly Anabaena flos-aquae), Romeria leopoliensis (formerly Synechococcus leopoliensis), Microcystis aeruginosa
Typha latifolia	Steroids, fatty acids	Triterpenes and steroids	Dolichospermum flosaquae (formerly Anabaena flos-aquae), Romeria leopoliensis (formerly Synechococcus leopoliensis), Microcystis aeruginosa

Globally, the highest inhibition effect of those compounds on cyanobacteria could mainly be caused by high concentrations of phenolic compounds (in addition to the presence of special phenolic compound or other allelochemicals and secondary metabolites that are still unknown. And the highest yield of allelochemicals extraction is permit with polar solvent.

Also, the sensitivity of cyanobacteria and phytoplankton to allelochemicals depending on abiotic and biotics parameters (Bauer et al., 2012) or the different physiological target processes (Hilt & Gross, 2008). In addition, the origin and the evolutionary history of the target algae species can also influenced their sensitivity (Callaway & Ridenour, 2004).

Most of macrophytes contain two active fractions (biomolecules of interest). One of them is hydrophilic, the other is moderately lipophilic (Gross et al., 2003). We can assume the presence of other allelochemicals, but for now, they are still unknown.

For now, we don't precisely know the nature of the active compounds, but we can assume that lipophilic compounds should mostly act on the plant surface via direct contact whereas hydrophilic compounds, due to their high solubility in water, are mostly released into the water.

3. Regulations: how to put a new PPP on the European Market?

Plant protection products (PPPs) containing active substance. The security of the active substance is evaluated by the Member States, the European Food Safety Authority (EFSA) and the Commission of EU to be placed on the market and be used in a plant protection product.

This process takes place in several steps, described in several documents called the "pesticides package":
- under Regulation (EC) No 1107/2009
- 2009/128/CE
- 1185/2009
- 2009/127/CE2009

First of all, the precision of the fact that the product (= PPP) falls in the scope of the regulations (EC) Regulation No 1107/2009 is needed. (Verheugen, 2005) https://ec.europa.eu/food/system/files/2021-05/pesticides_ppp_app-proc_guide_scope_reg-1107-2019.pdf.

Then, once it is confirmed, the product (=active substance), must be evaluated according to the document "**Regulation (EC) No 1107/2009**" in order to be authorized to be placed on the European market as candidates for substitution.

Here described the Protocol : (https://ec.europa.eu/food/plants/pesticides/approval-active-substances_en).

A "Rapporteur Member State" (RMS) is one of the EU country that evaluate an active substance dossier. Before submitting an application for the first EU approval, the producer of an active substance can choose the RMS and should contact the national competent authority.

NB: An alternative is the co-rapporteur (co-RMS) system where two or more EU countries collaborate. For renewal of approval, a RMS and a co-RMS are assigned by the Commission as set out in Regulation EU 686/2012. Also, applicants may request general and non-committing pre-submission advice from EFSA for applications for first approval, for renewal of approvals.

Concretely, the RMS will validate the prerequisites, tests and study reports required for submission on a case by case basis before confirming the admissibility of the application.
The applications' dossiers submitted for the approval, the amendment of approval or the renewal of an approval of an active substance have to be submitted via IUCLID format, which allows to share them with Member States, EFSA and the Commission. Dossiers must comply with the data requirements set out in Regulation EU 283/2013 and its associated Communication, as last amended.

Approximately 6 months are required between the submission of the application and the response. Applicants and laboratories are requested to inform about the studies commissioned or carried out in order to substantiate the application as much as possible.

The minimum of one use of the substances in PPP must be safe for people's health, including their residues in food, for animal health and must not have any unacceptable effects on the environment.

The substances approved for the first time, under **Regulation (EC) No 1107/2009** (1) or granted by the renewal of approval (2), are listed in Part E of the Annex to Implementing Regulation (EU) No 540/2011. For each active substance, the decision-making process prepare a review (1) or a Renewable Report (2), that provide details of decision and the criteria of evaluation, available in the database.

According to the applicant's dossier, the rapporteur Member State (RMS) and where relevant the co-rapporteur Member State (co-RMS), prepares a Draft Assessment Report (DAR) for first approval or a draft Renewal Assessment Report (RAR) for renewal of approval.

Based on the legislation, the RMS has to submit its assessment to the Commission and to EFSA one to one-and-half year after the admissibility of the application for a first approval or 13 months for a renewal of approval. EFSA then launches the peer-review process starting with a public consultation on the assessment prepared by the RMS (and co-RMS).

There is a common structure for the DAR/RAR agreed by the EU and OECD. The template can be found on the guidelines webpage.

In total, 3 years and 7 months are generally necessary to do all the procedures.

Once active substances are approved, companies can submit in the Member States applications for authorisation for placing on the market and use of plant protection products containing them.

(1) :

Regulation (EC) No 1107/2009 defines the procedures and criteria for the authorization of PPPs on the European market. It is necessary to meet these criteria detailed in the different chapters to be able to introduce a new PPP on the market (Europ & Pr, 2013).

(2) :

The initial approval of an active substance is valid for a limited period and the approval of an active substance needs to be reviewed periodically (The agreement is first given for a period of 10 years, (article 5 of the REGULATION (EC) No 1107/2009) and must be reviewed for a period not exceeding 15 years (article 14 of the REGULATION (EC) No 1107/2009). Be careful, if the active substances is concerning by the Article 4 of the REGULATION (EC) No 1107/2009 shall be for a period not exceeding five years (article 14 of the REGULATION (EC) No 1107/2009)).

Finally, note that renewals of approvals applications must be submitted at the latest 3 years before the expiry of the current approval of the active substance.
The details of the renewal procedure are set out in Commission Implementing Regulation (EU) No 2020/1740.

Conclusion

The main pests targeting the vineyards are classified as fungi. As we can see in the first part of the report, the diseases are different but can sometime be countered by the same molecule. Many researchers have studied a way to counter those pests and their different symptoms by using a handful of biomolecules from different plants.

We can note that:

- Alkali treated saponins coming from quinoa husk and Stilbene phytoalexins from Vitacae are useful against *botrytis cineria*'s fungi (only *in vitro*).
- Spraying seaweed extract on plants can fight fungi like *botrytis cineria.*
- *Ochrobactrum sp.* is a bacteria that has antagonistic activities at least with the bacterias *botrytis cineria* and *plasmapora viticola.*
- Metabolites enriched with phenylpropanoïds have a major role in protecting the hop crop against downy mildew.
- *Trichordema*'s family, a hyperparasitic fungi, can prevent harmful fungi like *Cryptosporella viticola* (excoriose) from expending by overgrowing them.

Therefore, it is important to conduct tests on various species of harmful fungi and see how they react to different allelochemicals. *In vivo* tests are mandatory to expend the research now that the *in vitro* tests were successful. There is a need to push further these trials to test them in real conditions before they are sold on the PPP market.

As it was discovered, several group of allelochemicals have an antifungal property. For example, monoterpenes, coumarins, quinones and many more have an antifungal effect. However, there is no certain studies about use of these biomolecules against the vineyard pests.

Concerning the allelopathic interaction of macrophytes on the development of cyanobacteria, we are now able to say that the type of solvent used to make the extraction has an impact on the action of the same allelochemicals of a macrophyte on a cyanobacteria.
Secondly, as described in the table at the end, macrophyte species produce different types of allelochemicals, which do not have the same target cyanobacteria organisms.
Furthermore, the mode of action is different for each allelochemical molecule since it does not engage the same reaction mechanisms (ROS formation…) and metabolism (PSII of photosynthesis, etc...) as another on the same bacteria.

To conclude, *Typha Latifolia* is a plant, only found near fresh water or rivers, that produce steroids and fatty acids. It should have an impact on fungi *Botrytis cineria*. In other hands, in the macrophyte *Potamogeton crispus* we can find steroids, terpenes, and alkaloids, that can probably have an impact on fungi pathogens of vineyards.

Discussion

Nowadays, the knowledge of the allelochemicals of macrophytes, and their impact on surrounding organisms (cyanobacteria, bacteria...) is not very developed, that is why we can say that we do not know all the allelochemicals produced by all macrophytes, but scientific community is aware that other allelochemicals, unknown, may be involved.

The main limits of this bibliography subject is the lack of details on review, in addition to the unknown information. Indeed, the researcher don't always precise the conditions of experiments they led. For example they don't precise exactly what they spread on plants, the exact molecule used.

All those things slow us down and block us in the progress of this bibliography report. It is especially the case to link the research on vineyard protection and the allelopathy. Indeed, some experiments are stopped in vitro, some go furthermore in details. It is hard to have the certitude that found results can be adapted to reality…

Most of time, the experiments are lead *in vitro* and don't go as far as the field experiment. It could be interesting, to continue the research, to test some of the *in vivo* biomolecules and technics that were found to be useful against fungi in real situations on the vineyards. That would determine if the biomolecules can be used against the fungi to prevent the yield loss or if using them outdoor is less effective or totally useless.

List of figures and tables.

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