PHYTOCHEMICAL ANALYSIS, ANTIFUNGAL ACTIVITY AND ALLELOPATHIC POWER OF AQUEOUS EXTRACT OF *EUCALYPTUS* ON THE GERMINATION OF SOME WEEDS

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Abstract

Our work aims to study the effectiveness of different concentrations of aqueous extract of *Eucalyptus Camaldulensis* (0%, 20%, 25% and 30%) on three fungi (*Aspergillus niger*, *Aspergillus parasiticus* and *Aspergillus ochraceus*) of durum wheat (Simeto variety) thus stored the allelopathic effect of extracts of *Eucalyptus camaldulensis* on the emergence of seeds of weeds present in soil previously cultivated with cereals. The test was carried out by sampling the targeted soils which were treated by adding the powder of *Eucalyptus camaldulensis* on the one hand or by spraying the aqueous extract of the plant *Eucalyptus camaldulensis* and were compared with the controls (soil untreated or plant not sprayed).

The phytochemical examination indicates that the aqueous extract of *Eucalyptus Camaldulensis* contains substances such as: alkaloids, polyphenols and saponins which may be responsible for antifungal activity. The tests of the antifungal activity different concentrations of the aqueous extract of *Eucalyptus camaldulensis* on the three isolated strains have a very significant efficacy ($P < 0.001$) especially *Aspergillus Parasiticuspuis*, *Aspergillus ochraceus* and weak inhibition of *Aspergillus Niger* in particular the very visible inhibitory effect which differs from one species to another and from one concentration to another. In addition, the soils treated with the powder of the plant *Eucalyptus camaldulensis* caused an inhibition and a delay of the germination of the weeds with a rate of emergence of 7% which explains its inhibitory effect on the seeds of the weeds. While the aqueous extracts of the plant *Eucalyptus camaldulensis* by spraying cause the total death of weed plants raised (100%).

The phytochemical examination indicates that the aqueous extract of *Eucalyptus Camaldulensis* contains substances such as: alkaloids, polyphenols and saponins which may be responsible for antifungal activity. Tests for antifungal activity at different concentrations of the aqueous extract of *Eucalyptus Camaldulensis* on the three isolated strains showed very significant efficacy (P <0.001) especially *Aspergillus parasiticuspuis*, *Aspergillus ochraceus* and weak inhibition of *Aspergillus niger* in particular the very visible inhibitory effect which differs from one species to another and from one concentration to another.

In addition, the soils treated with the powder of the plant *Eucalyptus camaldulensis* caused an inhibition and a delay of the germination of the weeds with a raised rate of 7% which explains its inhibitory effect on the seeds of the weeds. While the aqueous extracts of the plant *Eucalyptus camaldulensis* by spraying cause the total death of weed plants raised (100%).

Key words: Aqueous extract, Allelopathy, Antifungal activity, *Eucalyptus Camaldulensis*, Phytochemical, Weed.

Introduction

Cereals and their derivatives constitute the staple diet in many developing countries, particularly in the Maghreb countries. The origin of the contamination of cereals by mold is difficult to specify (field, transport, place of storage, etc.) (Djouhri, 2018). Durum wheat has always occupied the first place with more than half of the area reserved for cereals at 49% (CSCLG, 2016).

Crop protection plays an essential role in ensuring the safety and suitability of food evidencing that diseases are probably the biggest constraint in increasing the production and overall yield of the crop and one of the major factor limiting their quality (Morcia *et al.,* 2015).

Plant diseases are caused by pathogens such as fungi, weeds, bacteria, nematodes, and viruses. Compared to other plant pests, fungi have the greatest impact on disease and loss of production. This includes the considerable losses of foliage and fruits and vegetables after harvest that result from decomposition due to fungal pathogenic fungi (Strange and Scott, 2005). More than 150 species of molds and filamentous yeasts have been reported in cereal seeds. Most molds are probably found on grain seeds as exterior contaminants (Christensen, 1982).

Several parasitic cryptogams affect cereals and cause yield loss of varying importance, such as: *Aspergillus*, *Alternaria*, *Penicillium* and *Fusarium*. The secretion of highly toxic secondary metabolites, by mycotoxinogenic fungi during their proliferation on stored cereals, constitutes a real danger for the health security of man and animals (Tantaoui, 1977).

Weeds have always been a problem for agricultural producers. Heavy losses in yields and crop quality result from competition from weeds (Buhler, 2005). The competition between weeds and crops for water, light, nutrients and growing space can have a direct negative effect on yield. These losses are estimated at 9.7% of world agricultural production and are in the order of 10 to 56% in Africa (Traore *et al*., 2009) in (Hannachi, 2010).

The intensive use of agrochemicals, especially fungicides in the control of seed-based fungi and herbicides in weed control; have been heavily criticized for the health risks associated with their use. The search for new prevention strategies against infections, fungal

diseases and weeds, has a major interest, on the one hand for food safety and consumer health and on the other hand, for the protection of the country's economy.

Due to the aforementioned considerations, it may be necessary to develop new management systems to reduce reliance on synthetic agrochemicals. Recent trends favor the use of alternatives derived from natural plant extracts to control diseases and weeds. The use of plant extracts as a natural product is strongly encouraged because these products have many advantages such as reliability and ease of integration in practices not harmful to health and causing no pollution and the plants are available locally.

Thus, the uses of extracts from many species of plants have been reported to be effective against many fungi and weeds. Many know that higher plants produce economically important organic compounds, pharmaceuticals and pesticides (Amadi *et al.*, 2014). Plant extracts have the advantage of being not only inexpensively available to farmers, but also non-toxic and easily biodegradable and therefore healthy for the environment.

Our research aims to study the effect of the antifungal activity of aqueous extract of *Eucalyptus Camaldulensis* on three strains of *Aspergillus* (*Aspergillus Niger*, *Aspergillus parasiticus* and *Aspergillus Ochraceus*) as well as to test the allelopathy power of aqueous extract of species *Eucalyptus* plant on the germination and development of the main weed species.

Materials and Method

Biological material

To carry out this work we used three strains of *Aspergillus* isolated from the seeds of stored durum wheat of the simeto variety (Sersou).

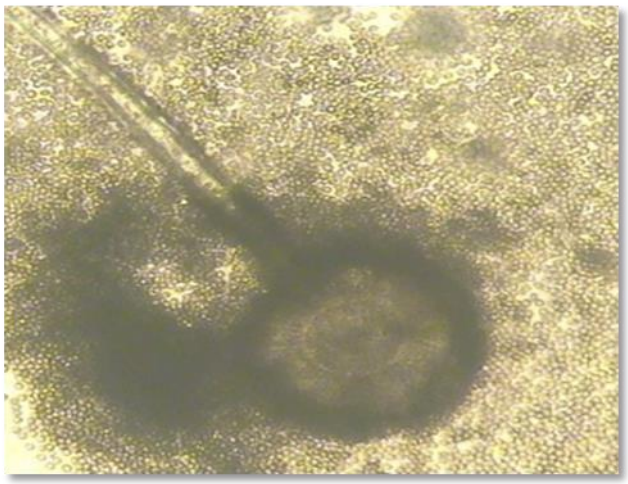


Fig. 1: *Aspergillus niger* under microscope (Grx 400)

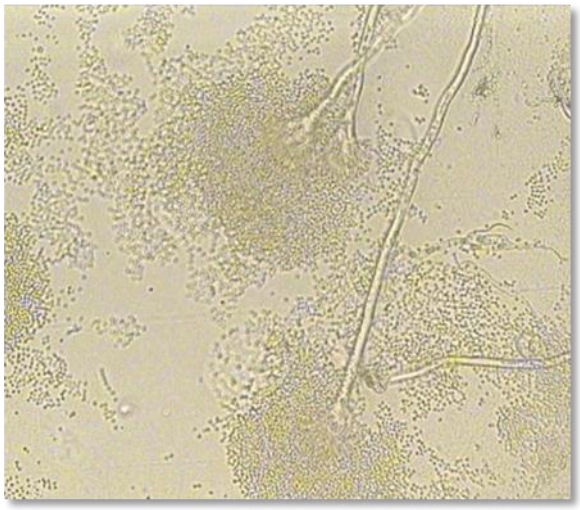


Fig. 2: *Aspergillus ochraceus* under microscope (Gr x 400)

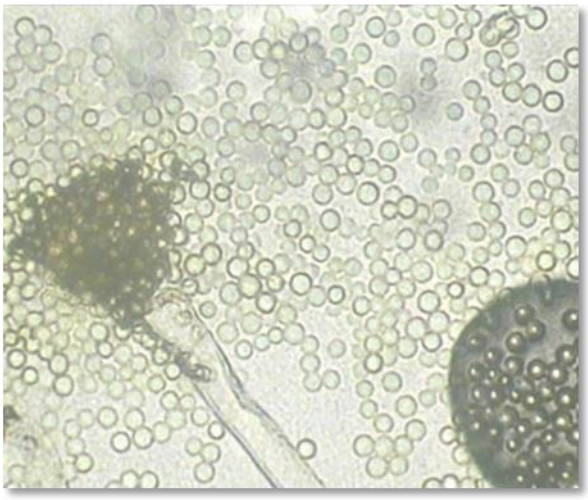


Fig. 3: *Aspergillus parasiticus* under microscope (Grx 400)

Preparation of the spore suspension

For the conservation of the isolates we also prepared spore suspensions, It consists in the inoculation of a few spores of a young culture in eppendorf tubes containing a semi-solid suspension based on 0.2% agar and a few drops of Tween 80. From this suspension, the identification of the genus and species is facilitated (Pitt, 1973 and Ramirez, 1982).

Allelopathic activity of aqueous extracts of *Eucalyptus camaldulensis*

Soil sampling

In order to take soil samples, we organized an outing during the period of February 2020 on a cereal pivot with an area of 14 hectares which is not planted this year (2020) in the Sebseb Wilaya valley of Ghardaïa. Simple random sampling was carried out at Cinque (5) different sites on the cereal pivot using an auger to a depth of 25 Cm.

Plant material

We have chosen the *Eucalyptus camaldulensis* which is commonly called in Algeria "Kalutous", and in several regions which is called kafor. The genus *Eucalyptus* is endemic to Australia and Tasmania. It is cultivated nowadays in some subtropical regions of Africa, Asia (China, India, and Indonesia) and South America as well as in southern Europe and the United States (Bouamer, 2004).

Eucalyptus camaldulensis belongs to the Myrtaceae family of dicotyledonous plants, it is distributed in about three thousand species distributed in 134 kind approx.

It is a woody plants of all sizes, from the shrub to the giant of the vegetable kingdom, reaching 100 meters in their countries of origin (approximately 40m for *Eucalyptus globulus*, approximately 25m for *Eucalyptus radiata*) rapid growth (several meters per an) Evergreen trees (Sandrine, 2006) found in the memory of (Mehani, 2015).

It was from 1860 that the *Eucalyptus* was introduced by the French in Algeria, along with the *Eucalyptus camaldulensis* Dehn. as a pioneer species. But, the massive planting of these trees took place between 1865 and 1963 early. *Eucalyptus* trees have been planted exceptionally for the draining of marshes (Mehani, 2006).

It was around the 1960s and 1970s that reforestation based on *Eucalyptus* began in the east of the country (EL-Kala, Annaba, Skikda) in the center (Tizi-ouzou and Bai nem) and in the West (Mostaganem) in order to meet national needs in wood products and with a capital of around 130 species. Planting of *Eucalyptus* continued until 1982 when the production of plants in the nursery and consequently their planting was ended (Mehani, 2006).

Eucalyptus are also extremely interesting for their tannins, resins and essential oils contained in the leaves, stems and even the bark and which have very important applications in medicine (Bigendako, 2004).

Systematic of *Eucalyptus camaldulensis*

The scientific classification carried out by the AGP (Angiospermes Phylogeny Groupe) on the genus *Eucalyptus* has made it possible to determine the following systematics (Guignard, 2001).

Reign: Planta
Under reign: Angiosperms
Class: Eudicots
Order: Myrtales
Family: Myrtaceae
Genus: *Eucalyptus*
Species: *Eucalyptus camaldulensis*

Preparation of aqueous extracts

For the present study, the leaves of the freshly harvested medicinal plant were dried in protection from light and moisture at room temperature. They are kept in clean bags to remove all impurities so that they are finely ground by an electric grinder.

Preparation of aqueous plant extracts

Regarding the extraction, we have adopted the maceration method which consists of leaving a solid in a liquid to stay cold to extract the constituents soluble in this liquid at a rate of 10% for 4 hours with stirring at 200 rpm. The mixture is then centrifuged at 3600 RPM for 30 min. the supernatant is recovered and then filtered through Wattman N^{o} 1 filter paper. This operation is repeated four times. At the end of the extraction, the extract obtained collected in a flask and stored at 4 °C protected from light until the time of their use (Razak *et al*., 2009).

Determination of the yield of dry extracts

According to Djeneb *et al*. (2016) the yield is the amount of extract obtained from the vegetable powder. It is expressed as a percentage or without a unit this results in the following formula:

$$r = (mx100)/M$$

r: extraction efficiency
m: mass of the extract
M: mass of plant powder

Phytochemical test

These are techniques that make it possible to determine the different chemical groups contained in a plant organ. These are physico-chemical reactions that identify the presence of chemicals. The phytochemical groups are numerous, but we can cite the main ones: alkaloids, polyphenols (flavonoids, anthocyanins, and tannins), saponosides, steroids, coumarins, sterols, terpenes…etc.

Detection of polyphenols

Tannin detection

The tannins are demonstrated from 1 ml of extract placed in a tube in the presence of a few drops of FeC13 (1% prepared with methanol). After stirring the extract, the color turns to black blue in the presence of gallic tannins and to greenish brown in the presence of catechetical tannins (Karumi *et al*., 2004).

Coumarin detection

Coumarins are revealed from 5 ml of extract placed in a tube brought to the boil until a volume of 1 ml is obtained; this volume is added to 1 ml of hot water. After stirring, the total volume is calculated into two volumes, one serves as a control and the other is added to 0.5 ml NH4OH (10%) and then examined under a UV lamp. The fluorescence emission indicates the presence of coumarins (Bruneton, 1999).

Detection of anthocyanins

Anthocyanins are detected by placing 5 ml of extract in a tube to which is added 15 ml of H2SO4 at (10%) (Acid medium), after stirring, the mixture is added to 5 ml NH4OH at (10%) (Medium basic). The presence of anthocyanins is confirmed by a blue-purplish coloration in a basic medium (Bruneton, 1999).

Flavonoid detection

A mixture of a few drops of Mg2 + and drops of concentrated HCL, placed in a tube, is added to 2ml of the extract. The appearance of pink, orange or red coloration indicates the presence of flavonoids (Malec and Pamelio, 2003).

Detection of anthraquinones

For the detection of anthraquinones, 10ml of extract is added to 5 ml of NH4OH (10%). after shaking, the appearance of a red ring indicates the presence of anthraquinone (Oloyede, 2005).

Saponosides detection

For the detection of saponosides, 1 ml of extract placed in a test tube is stirred for 15 seconds and then deposited for 15 minutes. A persistent foam height, greater than 1 cm, indicates the presence of saponosides (Koffi *et al.,* 2009).

Detection of terpenes

Steroid detection

The steroids are revealed after adding 5ml of acetic anhydride to 5 ml of hot extract. The mixture is added 0.5ml of concentrated sulfuric acid. After stirring, the appearance, at the interphase, of a purple or violet ring, turning blue then green, indicates a positive reaction (Bruneton, 1999).

Alkaloid detection

Alkaloids have been characterized using Mayer or Wagner reagents. 10 ml of the extract is evaporated until a volume of 20 ml is obtained, to which 1.5 ml of HCL (2%) are added. After stirring the acid solution, 1 to 2 drops of Mayer's or Wagner's reagent are added. The appearance of a yellowish-white or brown precipitate indicates the presence of alkaloids (Mojab *et al*., 2003). All the experiments are carried out in triplicate to check the reproducibility of the results.

Antifungal test

Preparation of different concentrations of aqueous extracts

We prepared four (04) concentrations of aqueous extracts (AE) adjusted using PDA, the four concentrations are prepared as follows in Table 1:

Table 1: Preparation of the different concentrations of aqueous extract

Concentration	**Witness 0%**	**20%**	**25%**	**30%**
PDA %	100 ml	80 ml	75 ml	70 ml
Aqueous extract (AE) %	0 ml	20 ml	25 ml	30ml

Preparation of pre-cultures

To perform the antifungal test, we adopted the direct contact method. In this step we used PDA and mixed it with lactic acid because molds can metabolize lactic acid but bacteria and yeast will be inhibited.

After preparations and sterilization of the PDA in the autoclave we added 25% lactic acid in proportion to 5 ml per 100 ml of PDA, and then we made the trilateral relationship for the other concentrations as follows: for 80 ml of PDA 4 ml of lactic acid, 3.75 ml for 75 ml of PDA and 3.5 for 70 ml of PDA are added. The addition of lactic acid is done before sterilization, to obtain at the end a sterile acidified PDA mixed with the different concentrations of the aqueous extract (sterile acidified PDA + Eq) which was poured into the 60 mm petri dishes. . After solidification we applied inoculation with spore suspensions of the three species (*Aspergillus Niger*, *Aspergillus Ochraceus* and *Aspergillus parasiticus*) Followed by incubation for 15 days at 25 °C in the oven.

The preparation of the different concentrations of the aqueous extracts of Eucalyptus *camaldulensis* is presented in the following **fig. 4**:

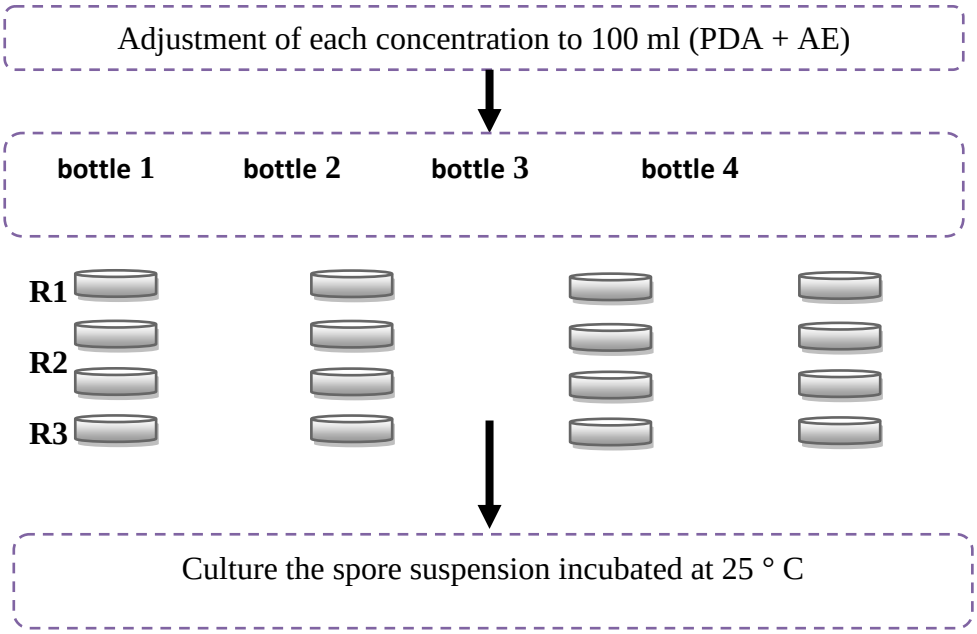


Fig. 4. Preparation of the different concentrations of aqueous extracts of *Eucalyptus camaldulensis*

Expression of antifungal test results

To assess the effect of the aqueous extract on the identified fungal strains we have chosen certain parameters and macroscopic and microscopic criteria such as:

Radial growth

Mycelial growth was assessed every 24 hours by measuring the average of three perpendicular diameters passing through the middle of the Round slice. Four repeats were performed for each concentration. This reading is always carried out in comparison with the control cultures that they are started on the same day and same conditions.

Mycelial growth kinetics

The mycelial growth kinetics corresponds to the variations over time in mycelial diameter under the effect of different concentrations of aqueous extract. The mycelial growth kinetics was evaluated every 24 hours by measuring the average of three perpendicular diameters passing through the middle of the disc. This reading is always carried out in comparison with the control cultures under the same conditions.

Determination of mycelial growth rate (VC)

According to Cahagnier and Molard, (1998) the rate of mycelial growth of each concentration is determined by the following formula:

$$VC = [D1/Te1] + [(D2-D1) /Te2] + [(D3-D2) /Te3] +\ldots+ [(Dn - Dn-1) / Te\ n]$$

D: Diameter of the growth zone for each day (mm)
Te: Incubation time (day)

Determination of the final diameter

The mycelial growth was evaluated at the end of the experiment, that is to say after the stop of growth of the control or the control fills the Petre dish, by measuring the average of three diameters without taking into account the diameter of the disc. This reading is always carried out in comparison with the control cultures which started on the same day and under the same conditions.

Inhibition rate

According to Leroux *et al*. (1978), the percentage inhibition of mycelial growth of the fungi tested is calculated according to the following formula:

$$Ic\ (\%) = (D0-Dc)/D0x100$$

D0: Diametrical growth of the witness
Dc: Diametrical growth of the fungus in the presence of a concentration (c) of the fungicide.

2.6.2. Spore production test

The method used to detect inhibition of sporulation is determined by the method of Leroux (1978). First, we must take a fungal disc from the center of the colony of each Petri dish then using the tips we transfer the disc into an Eppendorf containing 1 ml of sterile physiological water, we shake for 10 min to dislodge the spores. The number of spores was determined using a malassez cell, the unit of concentration (spore / ml). The percentage reduction in sporulation caused by plant extracts was calculated by the following equation:

$$Is = (No – N) /No\ x100$$

No: Average number of spores estimated in the control
N: Average number of spores estimated in the presence of the extract

Spore germination test

In order to perform the spore germination test and to examine the effectiveness of the aqueous extracts of *Eucalyptus Camaldulensis* with the three concentrations 20%, 25% and 30%, the method of Ahila Devi *et al. (*2004) was adopted, the method of Ahiladevi *et al.* (2004), Mlaiki (1970) was adopted with some modifications: The control sporal suspension collected from the spore production test is adjusted by diluting a mixture of one drop of the suspension spore of each fungal strain at 10^6 spore / ml with 10 ml of sterile distilled water. Three repeats of the spore suspension with the aqueous extract were performed simultaneously by each concentration. For the control, incubate at 25 °C for 24 hours and in the dark 500 µl of the spore suspension with 500 µl sterile distilled water.

Placed in a clean glass slide and inside the micro-humidity chamber (Petri dish contains a moistened filter paper) 50 µl of each mixture spread on slides covered with PDA medium. For germination of conidia Slides were examined at regular intervals by a microscope. The percentage count of germinated or non-germinated spores incubated at 25 °C and in the dark for 24 hours is determined under a microscope. A spore is considered germinated if the length of the germ tube is greater than the smallest spore diameter (Mlaiki, 1970).

We compared the germination percentage for each concentration by the following formula (Amadi *et al*., 2014):

(Number of germinated spore / total number of spores) x 100

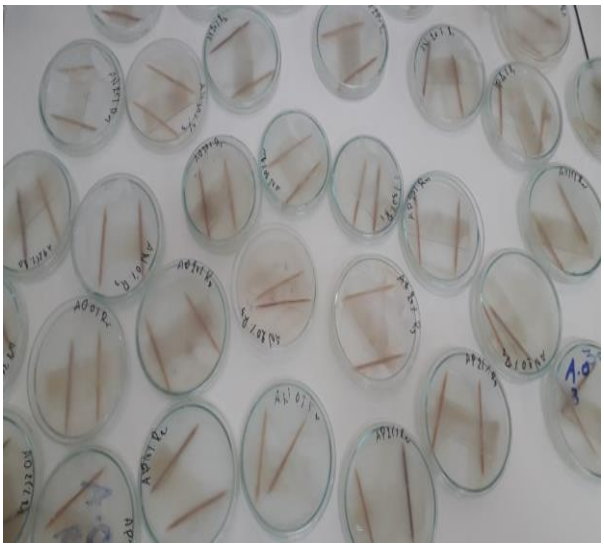


Fig. 5. Incubation of the spores in humid medium for 24 h at 25C °

Statistical analysis

The relative data from each trial was subjected to a one-way, two-way analysis of variance (ANOVA) (CoStatverion 6.4 software). An LSD test is performed if its requires ranking of the means, the values of $p < 0.05$ are considered significantly different.

Experimental apparatus

To test the effect of the powder and the aqueous extract of the plant *Eucalyptus camaldulensis* by spraying on the emergence of existing weeds in the soil planted by cereals, we took 15

aluminum boxes with a dementia of 18 cm of languor and 13 cm for the width of which each box we mixed 100g of potting soil with 200 g of sifted soil with three soil treatments for the five repetitions of each treatment including:

- First treatment (Control): (soil + compost).
- Second treatment (T1): 10 g powder of the *Eucalyptus camaldulensis* plant per 100 g of soil + potting soil.
- Third treatment (T2): spraying of an aqueous extract of the *Eucalyptus camaldulensis* plant after the emergence of the weed seedlings for 100g of soil + compost

This weight value of 100 g of compost with 100 g of sieved soil is used to determine the retention capacity of this substrate. This water characteristic is necessary because it makes it possible to calculate the quantity of running water supplied during watering and to calculate the retention capacity according to the following method:

$$\mathbf{C1= (P2-P1) = (112-100) =12\ g}$$

P1: Weight of the cultivated soil in 100g
P2: Weight of cultivated soil after 24h of saturation: 112g
12 ml: Retention capacity for 100 g of cultivated soil.

The experimental device of our study is represented in the following figure N6:

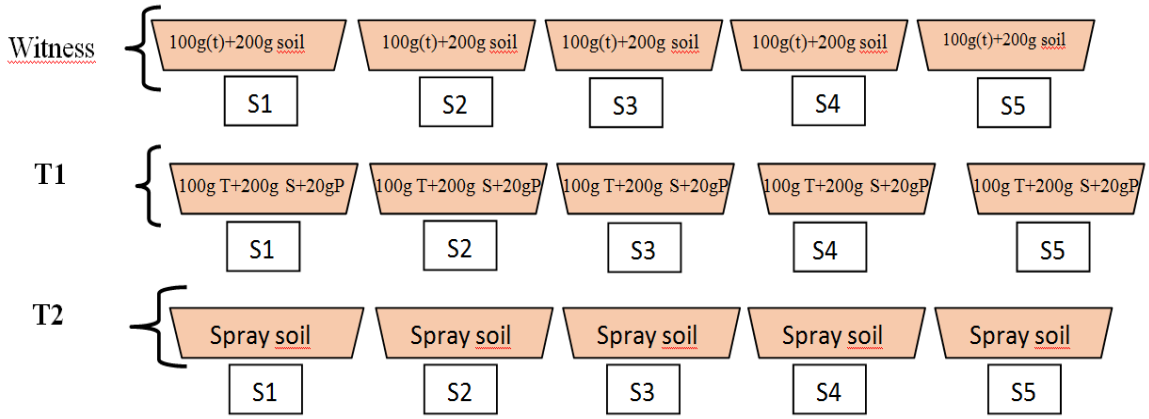


Fig. 6. Experimental setup

(T: treatment, S: soil, T1: powder treatment, T2: extract treatment, t: Soil p: powder)

Physiological parameters studied

For the present study, six physiological parameters are studied including: the maximum rate of germination of seeds of weeds, the maximum rate of inhibition of seeds of weeds, length of stems and roots of weed seedlings, fresh weight and dry weight of weed seedlings and the number of leaves of weed seedlings.

Maximum number of emergence of weed seedlings

The number of emergence of weed seedlings corresponds to the maximum count of seeds of sprouted weeds in all boxes during (21 days) of the experimentation period.

Germination kinetics

The germination kinetics corresponds to the variations over time in the emergence rate of control seeds and seeds treated with *Eucalyptus camaldulensis* powder and the aqueous extract.

Inhibition rate (Ti)

To determine the effect of aqueous extracts of *Eucalyptus camaldulensis* on the germination of the tested weeds, we will convert the percentage of germination to the percentage of inhibition. The conversions are carried out according to the formula Used by Dhima *et al*. (2006) and Chung *et al*. (2003).

$$TI\ \% = \frac{[(Witness - Extract)]}{100}$$

Whose:

TI% = (nbr seeds emerged in control - nbr seeds emerged in treated box / nbr seeds emerged in control) x100

Germination speed

The speed of germination Corresponds to the coefficient of the speed of germination (CVG) (Ranal and DE Sautana, 2006), It is noted as follows:

$$CVG = 100\ (n1+n2+\ldots\ldots+nx)\ /n1t1+n2t2+\ldots\ldots+nx\ tx$$

CVG: Germination rate
nx: Number of seeds raised for an observation.
tx: Day corresponds to the emergence of seeds.

Results

Yield of aqueous extract

The yield of the aqueous extract of the medicinal plant *Eucalyptus camaldulensis* is 59.1% and the pH reading is 4.50%.

Phytochemistry test

Phytochemistry screening of aqueous extract of *Eucalyptus camaldulensis*

Table 2 shows the major chemical groups of the aqueous extract of Eucalyptus *camaldulensis*. Phytochemistry screening results reveal the presence or absence of a secondary metabolite group.

Qualitative phytochemical tests based on color reactions or precipitation by specific chemical reagents carried out on extracts made from the powder of *Eucalyptus camaldulensis*. This extract consists of five chemical groups (tannin, Flavonoids, Anthraquinones, Saponosides and Alkaloids).

Table 2. Phytochemistry test carried out on the aqueous extract of *Eucalyptus camaldulensis*

Groupe chimique		Presence
Polyphenols	**Tannin (catechic)**	+
	Coumarin	–
	Anthocyanin	–
	Flavonoïdes	+
	Anthraquinones	+
	Saponosides	+
Terpenes	**Stéroïdes**	–
Alcaloïdes	**Alcaloïdes**	+

(-): absence**, (+) :** presence

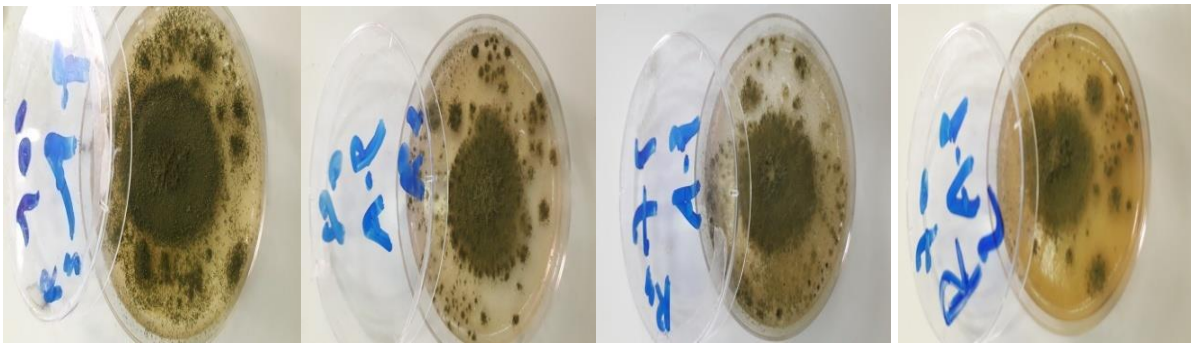
***In vitro* antifungal test**

Effect of the different concentrations of aqueous extract of *Eucalyptus camaldulensis* on the strains studied

The effect of the aqueous extract of the plant *Eucalyptus camaldulensis* on the studied fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ocharcuis* are shown in the following **Figure 7**:

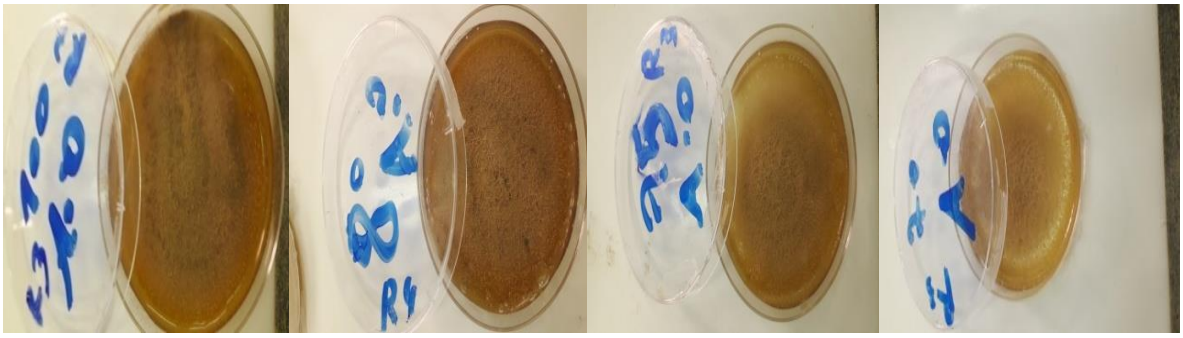
Aspergillus Parasiticus

T **20%** **25%** **30%**



Aspergillus Ochraceus

T **20%** **25%** **30%**



Aspergillus Niger

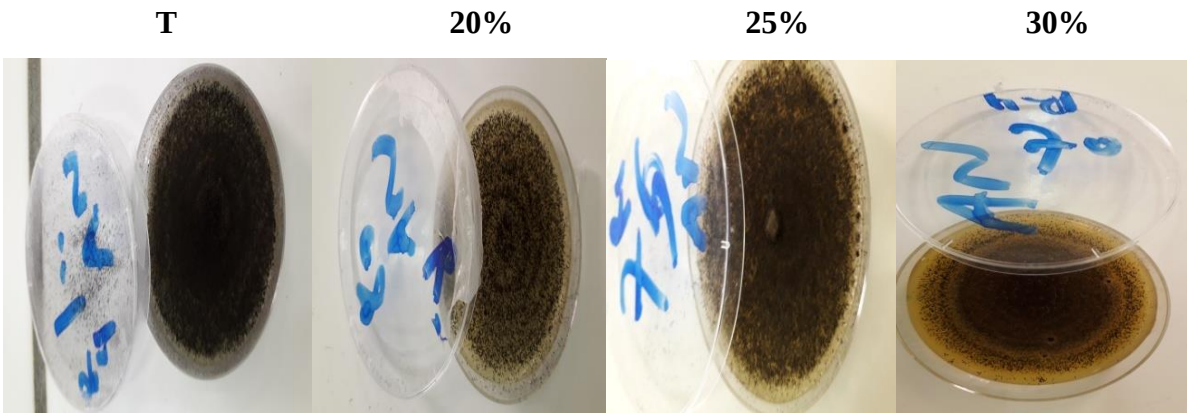


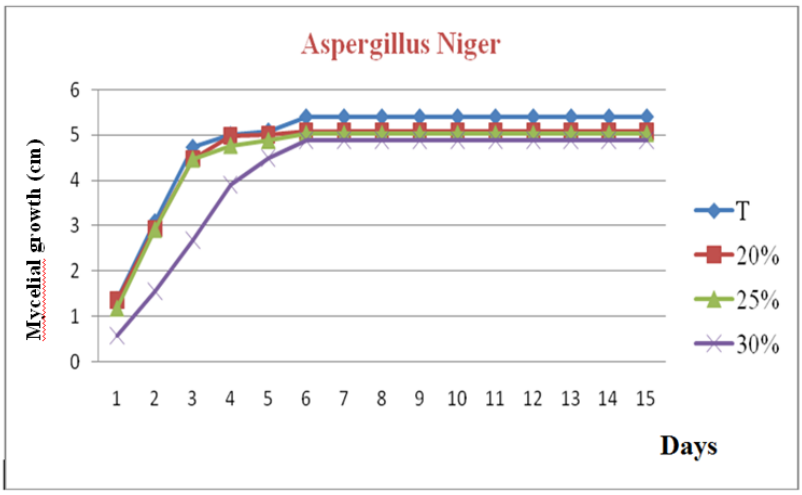
Fig. 7: Effect of aqueous extract of *Eucalyptus Camaldulensis* at different concentration on fungal strains

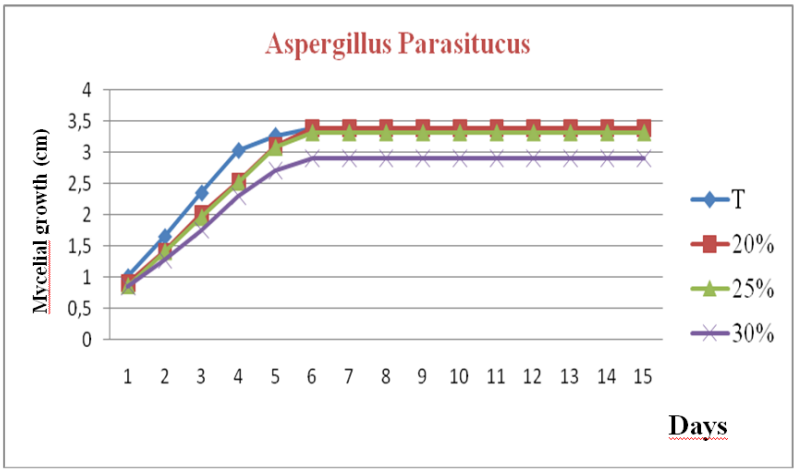
Fig. 7 represents the effect of the aqueous extract of *Eucalyptus Camaldulensis* on the morphology of the strains studied during incubation time (15 days), according to our results we notice that the effect of the aqueous extract *Eucalyptus camaldulensis* on fungi varied from one concentration to another and from one species to another.

The most important effect of aqueous extract of *Eucalyptus camaldulensis* is recorded with *Aspergillus Niger* with different concentration (control, 20%, 25%, 30%), we find that the effectiveness of aqueous extract of *Eucalyptus Camaldulensis* on fungal strains increase with increasing concentrations of aqueous extract of *Eucalyptus Camaldulensis*. Whereas, the control is more condensed with a very dark color compared to the other concentrations and we record the same results with *Aspergillus Parasiticus* and *Aspergillus Ocharceus*. The genus *Aspergillus* is a genus suitable for high concentration.

Mycelial growth kinetics of three fungal strains

The growth kinetics of the fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ocharceus* as a function of time and concentration of aqueous extract of the *Eucalyptus Camaldulensis* plant is shown in the following Fig. 8





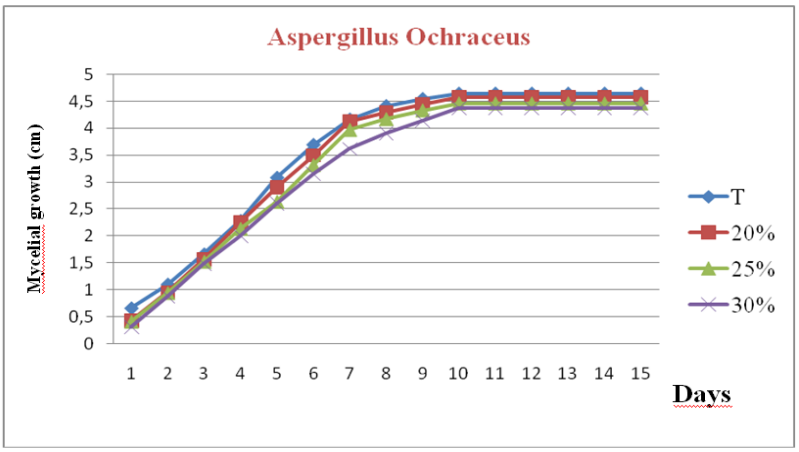


Fig. 8. growth kinetics of fungal strains as a function of time and concentration of aqueous extract of *Eucalyptus Camaldulensis*

According to fig.8 we notice that the highest mycelial growth of fungal strains is recorded in *Aspergillus Niger* with the different concentrations then in *Aspergillus Ochraceus* after in *Aspergillus parasiticus*. We observe that the mycelial growth of *Aspergillus Niger* was arrested with volume 5.4 in the control; on the other hand, the mycelial growth of *Aspergillus Niger* was inhibited from the 25% concentration. Stabilization of mycelial growth in *Aspergillus Niger* and *Aspergillus Parasiticus* was recorded in the sixth cheek, whereas in *Aspergillus Ochraceus* mycelial growth was inhibited on the tenth day.

This mycelial growth, which differs in concentration and species to another, is explained by the fungistatic effect of the aqueous extract of *Eucalyptus camaldulensis* which is variable according to the species and the concentration. The absence or presence of the mycelial growth revealed by the antifungal activity, with the different concentrations of aqueous extract of the *Eucalyptus Camaldulensis* plant on the different fungal strains. It is observed that the mycelial growth is remarkable and different after 24.

Determination of mycelial growth rate (VC)

The effect of different concentration of aqueous extract of the *Eucalyptus Camaldulensis* plant on the rate of mycelial growth of three fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ocharceus* are shown in the following Fig. 9:

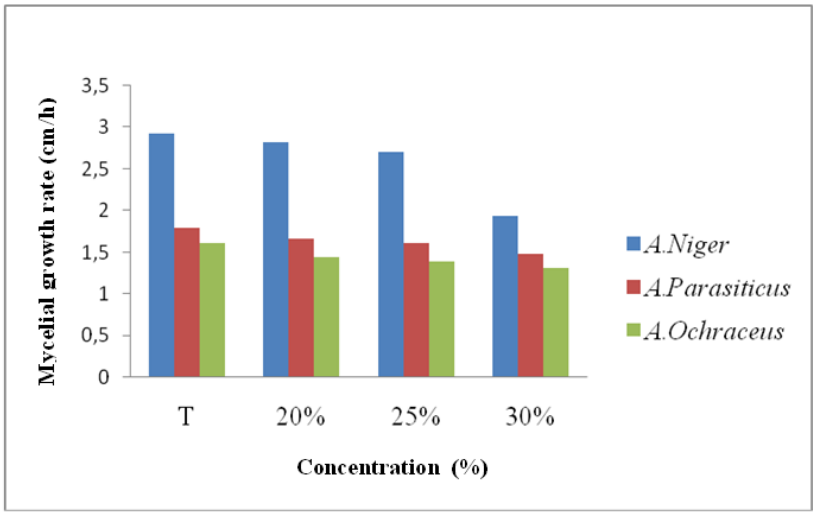


Fig. 9. Speed of mycelial growth under the effect of different concentration of aqueous extract of *Eucalyptus Camaldulensis*

According to Fig. 9 which shows the results of the speed of mycelial growth of the fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ocharceus* we observe a variation in values from one species to another. The witness of which recorded a faster rate of mycelial growth with the three fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ocharceus*. The latter decreases with increasing concentrations of aqueous extract of *Eucalyptus Camaldulensis*.

The speed of mycelial growth with the different concentrations is greater in *Aspergillus Niger* with the following values: 2.93cm/h, 2.82cm/h, 2.71cm/h, 1.94cm/h respectively compared to the speed of mycelial growth with the different concentrations in the two other fungal strains namely: *Aspergillus Parasiticus* which records the following values: 1.8cm/h, 1.66cm/h, 1.61 cm/h, 1.48 cm/h as well as *Aspergillus Ochraceus* which records the following results with the different concentrations: 1.61cm/h, 1.44 cm/h, 1.39 cm/h, 1.32 cm/h.

The greater speed of mycelial growth under the effect of aqueous extract of the plant *Eucalyptus Camaldulensis* is recorded in the fungal strain *Aspergillus Niger* compared to the two other fungal strains *Aspergillus Parasiticus* and *Aspergillus Ochraceus* which show a slower rate of mycelial growth

Determination of the final diameter (D)

The final mycelial diameter of three fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ochraceus* under the effect of aqueous extract of the plant *Eucalyptus Camaldulensis* are presented in the following Fg.10:

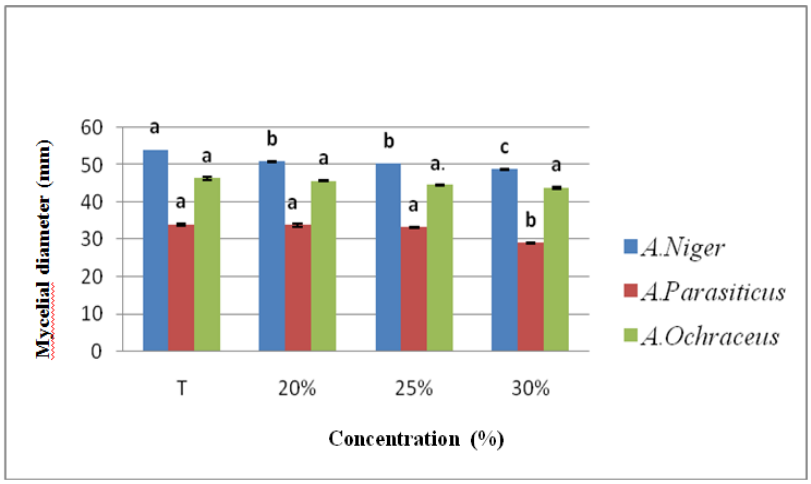


Fig. 10. Determination of final mycelial diameter of three fungal strains

We observe from fig.10, that the final mycelial diameter of fungal strains varies from one species to another, the largest mycelial diameter of which is recorded with the control in *Aspergillus Niger* with a value of 5.4 cm compared with the final mycelial diameter of two controls in *Aspergillus Ochraceus* and *Aspergillus Parasiticus* with the following values respectively: 4.64 cm and 3.39 cm, in addition the other concentrations register a weak inhibition compared to the control.

For *Aspergillus Niger*, the analysis of the ANOVA variance results shows a very highly significant effect between the treatments and the final mycelial diameter, The LSD test groups the treatments in (a) in the control, (b) that presents the treatment 20 % and 25% and (c) present the 30% treatment. For *Aspergillus Parasiticus* has a significant effect between treatments and final diameter, the LSD test groups the treatments in (a) in the controls, while the 20%, 25%, and 30% treatment groups in (b). In addition, *Aspergillus Ochraceus* after the analysis statistically shows an insignificant effect, and the LSD test groups the quarter treatments into group (a).

Inhibition rate (TI)

The inhibition rate of the three fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ochraceus* as a function of the concentration of the aqueous extract of the plant *Eucalyptus Camaldulensis* is shown in the following Fig. 11 after 15 days of incubation.

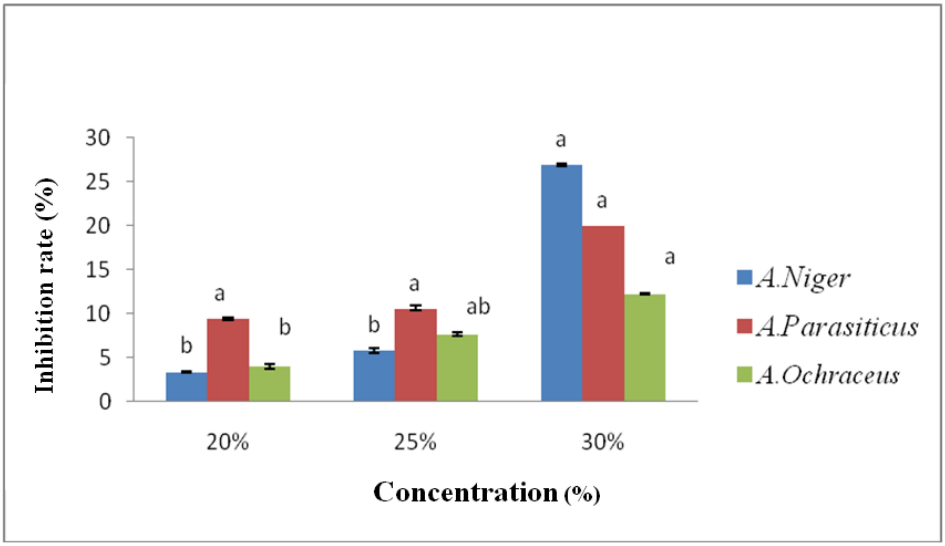


Fig. 11. Inhibition rate of fungal strains as a function of concentration of aqueous extract of *Eucalyptus Camaldulensis*

Fig. 11 shows the results of mycelial diameter inhibition of fungal strains under the effect of the aqueous extract of the *Eucalyptus camaldulensis* plant during the incubation time (15 days) we note that there is a different inhibitory activity of aqueous extract on the three fungal strains. The most significant inhibitory activity of which is recorded with the concentration 30% in the three fungal strains *Aspergillus Niger* (26.94%), *Aspergillus Paraiticus* (20%) and *Aspergillus Ochraceus* (12.25%), on the other hand the inhibitory activity is very low with the control in the three fungal strains. For *Aspergillus Niger*, the analyzes of the results statistically show a very highly significant effect between the treatments and the rate of inhibition of spore production, *Aspergillus Parasiticus* shows a significant effect and *Aspergillus Ochraceus* shows a non-significant effect.

LSD tests group together the treatment in (a) at the 30% concentration and in (b) group together the 20% and 25% treatment for *Aspergillus Niger*. In addition, *Aspergillus Parasiticus* combines the three concentrations in (a), for the concentration 20% in (b), 25% in (ab) and 30% in group (a).

Spore production (TS)

Fig. 12 represents the rate of inhibition of spore production of the three fungal strains *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ochraceus* under the effect of the aqueous extract of the plant *Eucalyptus camaldulensis* according to different concentration

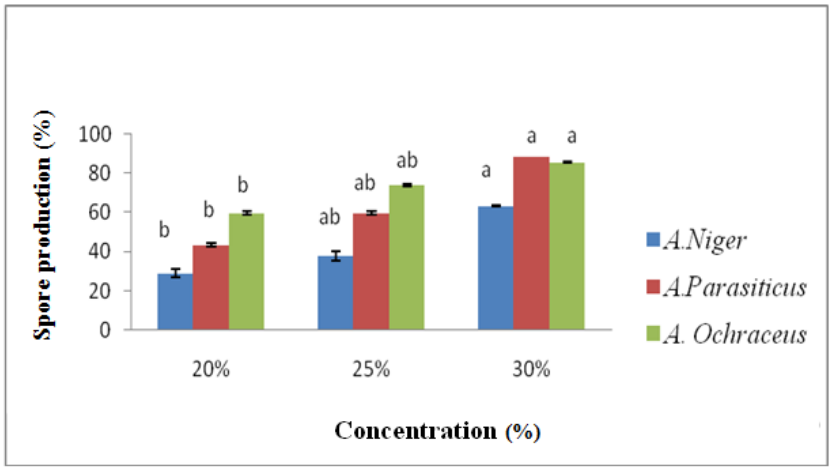


Fig. 12: Effect of aqueous extract of *Eucalyptus camaldulensis* at different concentration on the rate of inhibition of spore production of fungal strains

According to Fig. 12 which represents the rate of inhibition of the production of spore of three fungal strains under the effect of the aqueous extract of the plant *Eucalyptus camaldulensis* according to different concentration after 24 hours of incubation, we note that the inhibition of sporulation at the 20% concentration reaches a value of 59.81% in *Aspergillus Ochraceus* and an average rate of inhibition of sporulation in Aspergillus *Parasiticus*, on the other hand, it is low in *Aspergillus Niger* with a rate of 29.01% .

Moreover, with the 25% and 30% concentration we observe an inhibition of the production rate of the spores of fungal strains which are very strong in *Aspergillus Parasiticus* and *Aspergillus Ochraceus*. Thus in *Aspergillus Niger* shows a very strong inhibition at 30%, the rate of inhibition of spore production being 63.48% while it is weak at 25% with a rate of 37.88%.

Statistical analysis shows that there is a very highly significant effect between treatments and the rate of inhibition of spore production in *Aspergillus Nige* and an effect is very significant in *Aspergillus Parasiticus*, on the other hand a very highly significant effect in *Aspergillus Ochraceus*. The LSD test combines the treatment 30% in (a), 25% in (ab) and 20% in (b) in the three species studied.

Spore germination (TG)

The percentage of germination of spore of *Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ochraceus* under the effect of different concentrations of the aqueous extract of the plant *Eucalyptus camaldulensis* is shown in the following Fig. 13:

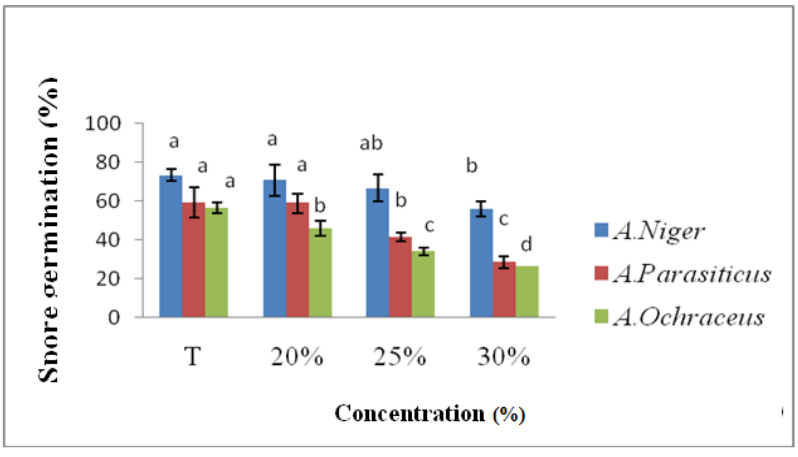


Fig. 13. Effect of different concentration of the aqueous extract of *Eucalyptus camaldulensis* on the spore germination rate of three fungal strains

According to Fig.13 we note after 24 hours and with the increase in the concentrations of aqueous extract of the plant *Eucalyptus camaldulensis* that the germination of the spores is very low especially with the concentration 30% for the three fungal strains, *Aspergillus Niger* 49%, *Aspergillus Parasiticus* 28%, and *Aspergillus Ochraceus* 26%. On the other hand, with the 0% concentration (control) of aqueous extract of the plant *Eucalyptus camaldulensis*, there is a variation in the germination values between the three fungal species, of which it is significant in *Aspergillus Niger* with a percentage of 73%, moderately in *Aspergillus Parasiticus* with a spore germination percentage which is 65% and a low spore germination percentage in *Aspergillus Ochraceus* which is 47%.

Statistical analyzes of the results show a significant effect between treatments and spore germination rate for Aspergillus Niger. In addition a very highly significant effect for *Aspergillus Parasiticus* and *Aspergillus Ochraceus*. The LSD test groups the treatment into two (a) in the control with the concentration 20%, (ab) in 25% and (b) in 30% in *Aspergillus Niger*. Whereas, the LSD test groups the treatment in (a) for the control, (b) in 20%, (c) in 25% and (d) in 30% for *Aspergillus Ochraceus*.

Effect of powder from the plant *Eucalyptus camaldulensis* on the germination of existing weeds in the soil planted by cereals

Weed germination kinetics

Fig. 14 expresses the kinetics of the germination of weeds existing in the soil previously planted by cereals over time treated with the powder from the leaves of the plant *Eucalyptus camaldulensis* and with tap water.

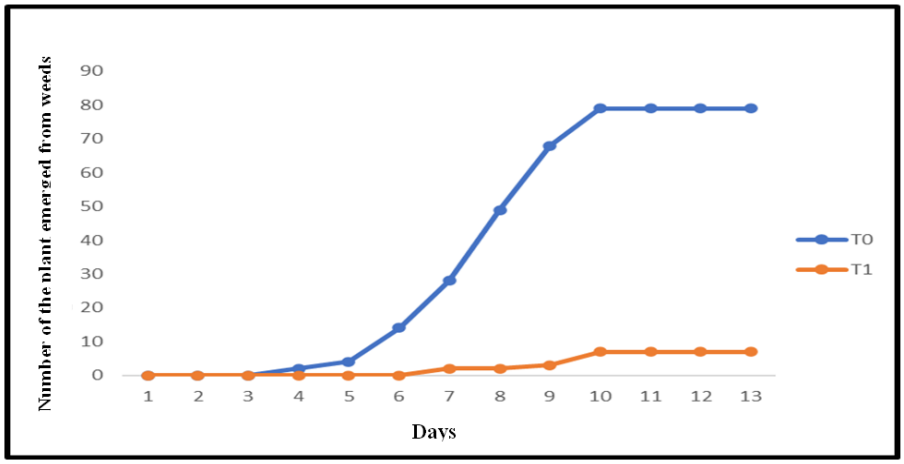


Fig. 14. Effect of *Eucalyptus camaldulensis* powder on the germination of the number of the plant emerged from weeds

T0: Treatment of the control soil without *Eucalyptus camaldulensis* powder
T1: Treatment of the soil with powder from the leaves of the plant *Eucalyptus camaldulensis*

According to the results obtained in Fig. 14 which shows the evolution of the effect of the powder of the plant *Eucalyptus camaldulensis* on the germination kinetics of the number of the plant emerged from weeds, we note that there is a variation in number of plants emergence of weeds, thus stabilization of the number of weeds emerged at control soil levels (T0) without powder of the *Eucalyptus camaldulensis* plant during the fourth day which begins to increase and stabilize after the tenth day with a number of lifting which is 79%. On the other hand, the soils treated with the powder of the plant *Eucalyptus camaldulensis* (T1) begin to rise from the seventh day with a number of 7 liftings.
It should be noted that the number of plants emerged is 79% obtained with the treatment of the control soil (T0) without powder of the plant *Eucalyptus camaldulensis*.

Total number of weed emergence
After 10 days of germination, we observe the results of the effect of soil treatment with powder of the plant *Eucalyptus camaldulensis* (T1) and by treatment of control soil (T0) without powder of *Eucalyptus camaldulensis* on the final number of the emergence of seeds of weeds stored in the soil which are shown in fig. 15

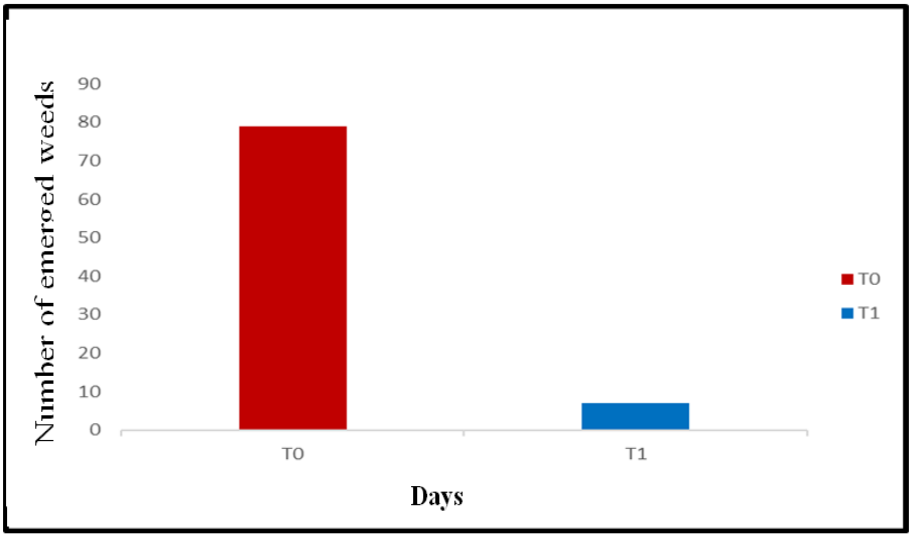


Fig. 15. Effect of *Eucalyptus camaldulensis* powder on the total number of emerged weeds

T0: Treatment of the control soil without *Eucalyptus camaldulensis* powder
T1: Treatment of the soil with powder from the leaves of the plant *Eucalyptus camaldulensis*

According to fig.15 which represents the effect of the powder of *Eucalyptus camaldulensis* on the total number of emerged weeds, we notice that the total number of emerged weeds treated with the powder of the plant *Eucalyptus camaldulensis* (T1) registers a low rate which is 7% compared to weeds germinated in the control soil (T0) treated without powder of the *Eucalyptus camaldulensis* plant with a percentage of 79%.

Percentage of weed emergence

Figure 16 expresses the effect of powder from the *Eucalyptus camaldulensis* plant on the percentage of seeds emerged from weeds over the course of days.

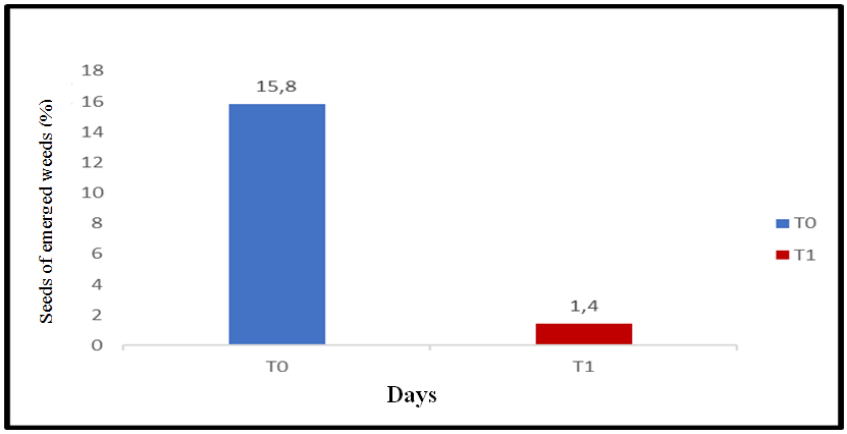


Fig. 16. Evolution of seeds of emerged weeds

T0: Treatment of the control soil without Eucalyptus camaldulensis powder
T1: Treatment of the soil with Eucalyptus camaldulensis powder.

According to the results obtained in fig. 16 which represents the evolution of seeds emerged from weeds, we record a low rate of seeds raised from weeds with the soil treated with the powder of the plant *Eucalyptus camaldulensis* with a percentage of 1.4%. In addition, the control soil treated without *Eucalyptus camaldulensis* powder recorded a high rate of 15.8% of emerged weeds.

Lifting speed (T50)

The results of Fig. 17 expressed the effect of the powder of *Eucalyptus camaldulensis* on the numbers of seeds emerged and on the emergence T50 of seeds of weeds of the soil (T1) treated with the powder of the plant *Eucalyptus camaldulensis* and control soil (T0) not treated with powder from the plant *Eucalyptus camaldulensis*

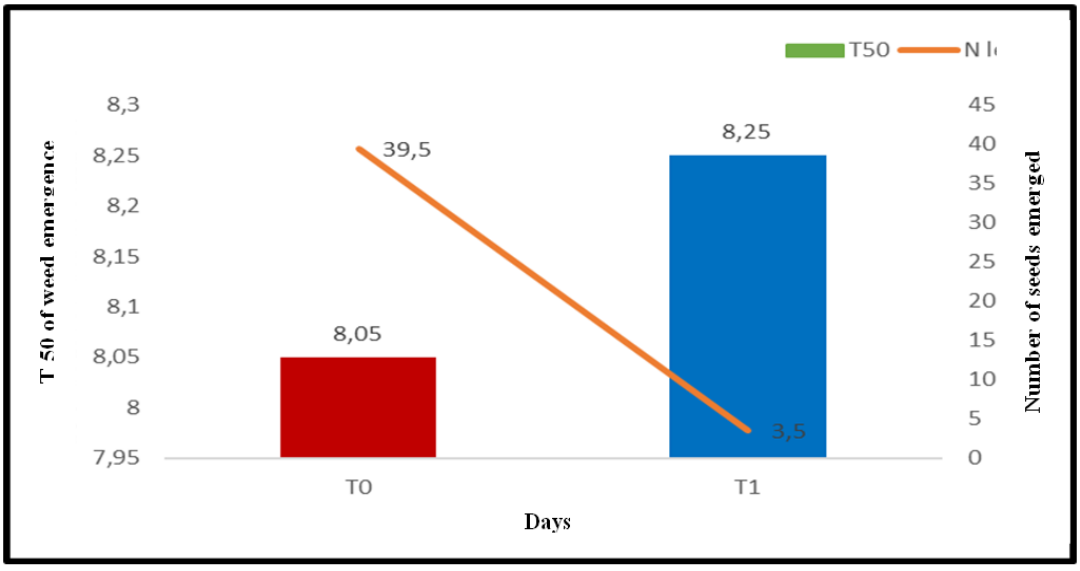


Fig. 17. Number of seeds emerged and T 50 of weed emergence

T0: Treatment of the control soil without *Eucalyptus camaldulensis* powder
T1: Treatment of the soil with *Eucalyptus camaldulensis* powder.

From the results obtained according to Fig.17, we see that there is a variation in the number of seeds emerged and T50 of emergence of seeds of weeds, including the number of emergence of seeds of weeds at soil level. Controls (T0) treated without *Eucalyptus camaldulensis* powder is 39.5% compared to soils treated with powder from the *Eucalyptus camaldulensis* plant (T1) which records a value of 3.5%.

While for the T50 of emergence of seeds of weeds, we record almost equal values between control soils control soils (T0) treated without powder of *Eucalyptus camaldulensis* and soils (T1) treated with powder from the plant *Eucalyptus camaldulensis* including the values respectively are: 8.05 and 8.25%.

Effect of aqueous extract of Eucalyptus camaldulensis by spraying on the germination of existing weeds in the planted soil

Weed mortality kinetics

Fig. 18 expresses the kinetics of weed mortality, which corresponds to the variations in the number of dead plants of weeds over time under the effect of the aqueous extract of the plant *Eucalyptus camaldulensis* by spraying.

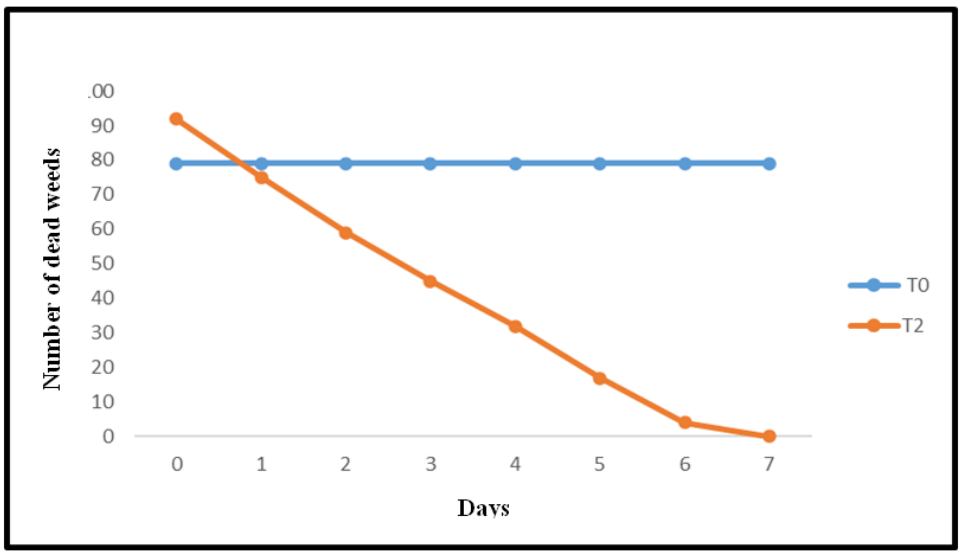


Fig. 18. Effect of aqueous extract of *Eucalyptus camaldulensis* by spraying on number of dead weeds

T0: Control treatment of weed seedlings without aqueous extract of *Eucalyptus camaldulensis*.
T1: Treatment of weed seedlings with aqueous extract of *Eucalyptus camaldulensis* by spraying.

From the results obtained in Fig. 18, We find that there is a variation in the number of dead plants of weeds, with wilting and death of all weeds after yellowing and decrease in germination rate in plants treated with the aqueous extract of the plant *Eucalyptus camaldulensis* (T1) by spraying from the 1st day until the 7th day of the treatment application with a mortality rate of 92%. In contrast, no sign of wilting or death of control weeds (T0) treated without aqueous extract of *Eucalyptus camaldulensis*.

Spray effect of aqueous extract of *Eucalyptus camaldulensis* on weed mortality rate

The results in Fig.19 express the effect of the aqueous extract of the plant *Eucalyptus camaldulensis* by spraying on the rate of dead weeds over time.

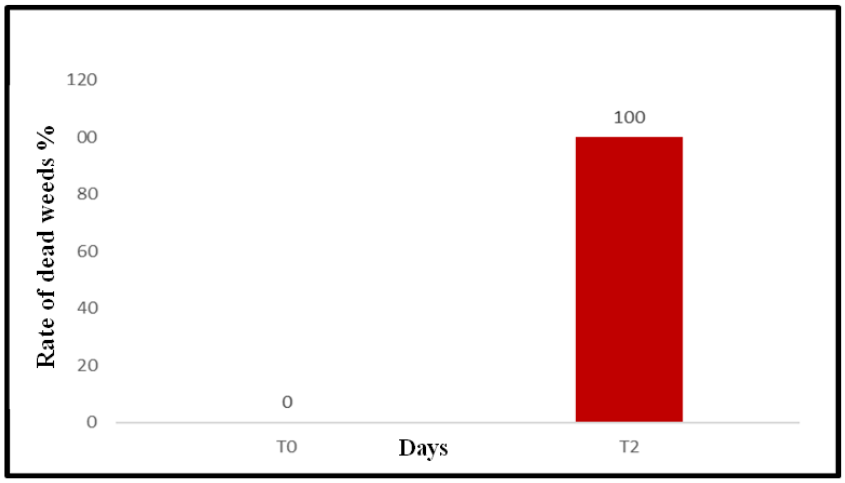


Fig.19: Rate of dead weeds

T0: Control treatment of weed seedlings without aqueous extract of *Eucalyptus camaldulensis*
T1: Treatment of weed seedlings with aqueous extract of *Eucalyptus camaldulensis* by spraying

According to the results recorded in Fig. 19 which represents the rate of dead weeds over time, we notice a high rate of death of seeds raised from weeds with the soil treated with the aqueous extract of the plant *Eucalyptus camaldulensis* by spraying with a rate of 100%. In addition, the control soil treated with tap water recorded a rate of 0% of dead weeds.

Discussion

Our work deals with the effect of antifungal activity on the three fungal strains of *Aspergillus* (*Aspergillus Niger*, *Aspergillus Parasiticus* and *Aspergillus Ocharceus*) of stored durum wheat of simeto variety as well as the Allelopathy power of aqueous extract of *Eucalyptus* plant species on germination and development of the main weed species. Many studies have noted the possibility of using the aqueous extract of the plant as an effective natural alternative.

The yield of aqueous extract of *Eucalyptus camaldulensis* is 59.1%, so this value is very high compared to the result of Djouhri (2018) which recorded a very low yield in

Artemisia herba alba from Djanet which is 18.33% and a yield of 16.33% of *Artemisia herba alba* from the Tébessa region.

This variation is linked to environmental factors such as climate (climatic ecotypes), the nature of the soil (edaphic ecotypes), the position of the species in a habitat or within an association (biotic ecotypes) or its location (geographic ecotypes). Indeed, Svoboda and Hampson, 1999 and Smallfield (2001) report that environmental conditions, timing, harvest and age of plant material can influence extraction yields. In addition, not all plant species have the same potential; some botanical families offering higher yields than others (Valnet, 1980).

The pH measurement of the aqueous extracts of *Eucalyptus Camaldulensis* gave an acidic pH (4.5) which is similar to the pH found by (Kemassi and Azouzi, 2017) which is 4,89.

Based on phytochemical tests the presence or absence of chemicals based on precipitation or staining by specific chemical reagents. According to the results of phytochemical tests of aqueous extract of *Eucalyptus Camaldulensis* we note the presence of tannins, flavonoids, anthraquinones, saponosides and alkaloids. Therefore, the presence of secondary metabolites confirms the presence of antifungal and herbicidal property in this extract which can be explained by the synergistic effect between the different extract compounds.

The work carried out by Kamassi and Azouzi (2017) and Djouhri (2018) reported that the plant extracts of plants of *Asphodelus tennifolius* and *Artemisia herba alba* contain chemicals such as tannins, alkaloids, saponosides…etc, the latter is responsible for the biological activity. According to Sofowora, (1996) the composition of a plant in secondary metabolites varies depending on the geographical location, the organ removed, the period, the time of collection and the storage conditions.

We know that plants synthesize different bioactive compound in plant tissues like alkaloids, flavonoids, tannins, terpenoids, saponins and other compounds with antifungal and herbicidal properties which stop or inhibit the development of growth or the germination of mycelia, reduce sporulation of pathogens such as fungal, bacterial, viral or weeds ... etc.

Bravo, (1998) notes that, polyphenolic compounds play an important role in growth and reproduction, providing protection against predators and pathogens. Saponins are also a special class of glycosides which have a soapy characteristic on the one hand and very good antifungal activity on the other. Sadipo *et al.* (1991) show that saponins have a wide range of antifungal activities. Thus the works of Scalbert (1991), Banso and Adeyemo (2007) and Bassou, (2007) have demonstrated that the tannins isolated from medicinal plants have toxic activity against fungi. According to Sadipo *et al*. (1991), tannins are a phytochemical substance having a major role in inhibiting the growth of microorganisms by precipitation of microbial proteins. Plants containing alkaloids have high nitrogenous organic constituents which can be attributed to their ability to become toxic and even adductive (Jacob et al., 2006).

The direct contact technique consists of bringing the aqueous extract of *Eucalyptus Camaldulensis* into contact with the three fungal strains of *Aspergillus* (*Niger, Ochraceus, Parasiticus*) then the observation of: growth, inhibitions, speed, production, and germination of these fungal strains.

Morphological changes (color of the mycelium, mycelial tears, diameters, sizes, etc.). This modification was observed in the different concentrations on the three fungal strains tested (*Aspergillus Niger*, *Aspergillus Ochraceus*, *Aspergillus Parasiticus*), but in different values, for example the volume, the color of the very dark controls compared to the other concentrations in the three species of fungi… etc. Our results are consistent with the results of (Kemassi and Azouzi, 2017).

Regarding the final mycelial diameter, it was large in *Aspergillus* Niger and medium important in *Aspergillus* Ochraceus because the two fungal strains are less sensitive to the aqueous extract of *Eucalyptus Camaldulensis*. This result is similar to the result of DJOUHRI et al., 2018. On the other hand, the final mycelial diameter of *Aspergillus Parasiticus* is small with all four concentrations. (Kemassi and Azouzi, 2017), noted that the growth of *Aspergillus Parasiticus* was present except in the control with the 20% concentration. Mycelial growth is noted in *Aspergillus Niger* because it is very resistant to harsh conditions. In general, species of the genus *Aspergillus* are resistant to the direct contact method. Our results are similar with the results of (Djouhri *et al*., 2018).

We note that the 30% concentration combines (c), 25% (b), 20% (ab) and 0% (a) therefore the mycelial growth decreases when the concentration of aqueous extract increases until the final stop of the growth. Our results are similar to the results of (Kemassi and Azouzi, 2017). Indeed, the inhibition of growths of fungal strains is different, because our results do not indicate a total inhibition. The different concentrations of aqueous extracts significantly influenced the radial growth of the fungus. The work of Euloge *et al.* (2013) demonstrated that the antifungal tests revealed that the plant extracts used have an antifungal activity whose intensity varies depending on the plant species, but also on the fungal strain tested.

Regarding the results obtained for the final diameter of mycelial growth shows that the least sensitive molds are *Aspergillus Niger* while *Aspergillus Parasiticus* is the most sensitive to the aqueous extract of *Eucalyptus Camaldulensis*.

The rate of inhibition of sporulation increased with increasing concentrations of aqueous extract, our results are in agreement with the results of Rafiqul et al (2003) and Amedi et al (2014) which showed that the reduction of sporulation was increased with increasing concentration of aqueous plant extracts. The inhibition of the sporulation of *Aspergillus Parasiticus* and *Aspergillus Ochraceus* by the aqueous extract of *Eucalyptus camaldulensis* is more important than the inhibition of the sporulation of *Aspergillus Niger*.

The difficulty of developing an antifungal molecule is linked on the one hand to the ultrastructure of the fungal cell which presents three barriers: the chitinous cell wall, membrane ergosterols and the eukaryotic nucleus (Chami, 2005), and on the other hand, the antifungal molecules themselves which can generate resistance (Prasad and Kapoor, 2004). The extracts caused significant inhibition in mycelial growth, sporulation and spore germination (Serghat *et al.*, 2004).

The experimental study of the effect of the aqueous and powdery extract of the plant *Eucalyptus camaldulensis* on the germination of existing weeds in the soil planted with the screen by cereals shows the existence of allelopathic phenomena in experimental conditions. Kruse *et al*. (2000) have shown that when sensitive plants are exposed to allelochemicals, seed germination is delayed.

The herbicidal effect of the aqueous extract of *Eucalyptus camaldulensis* at different spraying is very remarkable for all the soils which contain the seeds of the weeds tested. Regarding the rate of inhibition of germination of weeds gradually increases with the concentration of the extract. This is probably due to the high susceptibility of some weeds compared to others. Kruse *et al.* (2000) also showed that the effect of allelochemicals is manifested by morphological variations (lengthening of the stem and radicle) which are most often observed in the early stages of growth. In most of the tests that we have performed, the inhibitory effect of the extracts is greater on the growth of the seedlings (length of the radicle and length of the stem).

According to (Rsaissi *et al*., 2016) who show that Allelopathic substances can be exploited for the control of weeds and be used in the development of herbicides. Several researchers have also reported that *Eucalyptus* species are rich in allelopathic substances which may be effective for biological control (Bowman and Kirkaptric, 1936; Igboanugo (1936, 1937); Lovett, 1989) in (boudiaf, 2017).

The herbicidal activity of our extracts could be explained by the presence of abundant compounds such as terpene, tannic and flavonoid substances. In fact, it is known that the herbicidal compounds of natural or synthetic origin are inhibitors of cell division and in particular the blocking of tubulin and of the achromatic spindle (Calvet et al., 2005). They are also growth disruptors with an inhibitory action on the synthesis of auxin and auxin transport. They act negatively on decoupling, photosynthesis and on the synthesis of folic acid and cellulose (Calvet *et al.*, 2005).

Conclusion

With the advent of synthetic pesticides about 50 years ago, we thought about eliminating crop pests such as weeds. However, farmers with access to synthetic pesticides seldom suffer from devastating infestations and the increase in the quantity and quality of agricultural products is the cause of the excessive use of pesticides. However, in recent years and faced with increasingly restrictive legislation on the application of synthetic pesticides, the search for phyto-herbicides and fungicides has proved to be a strategy particularly suited to consumer requirements while preserving the 'environment. Indeed, international bodies such as the OMS have banned the use of certain chemically synthesized products such as organochlorines.

Our research work was based on the identification of plants likely to constitute a biological control accessible, effective and adapted to the cultivation of cereals, in particular, in cereal areas where direct sowing is practiced. For this we have chosen to research substances of natural origin which by their antifungal activities and which could be used to fight against fungi and weeds.

The established results give reasonable hope for the use of the aqueous and powdery extracts of the plant *Eucalyptus camaldulensis* as an inhibitor of fungal and weed germination. Indeed, *Eucalyptus camaldulensis* has been shown to be a powerful inhibitor, which confirms previous work which has demonstrated the allelopathic faculty of the *Eucalyptus* plant.

These results suggest that the aqueous extract of *Eucalyptus* could serve as a fungicide and a natural weedkiller accessible, effective and suitable for the cultivation of cereals at

suitable doses. The foliar extract of this plant (*Eucalyptus*) can replace synthetic chemical pesticides in the field of phytoprotection, especially since the *Eucalyptus* is relatively abundant and hardy. To this end, the plant heritage must be absolutely preserved in its diversity and in its extent.

In perspective, this study once again allows the development of the use of aqueous and powdery plant extracts as natural herbicides in agriculture with a view to preserving human health and contributing to the protection of the environment.

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