

# Effects of two fungicidal products (mancozèbe and azoxytrobine) and rosemary essential oil on *Lactuca sativa* physiology

FENNANE Amal<sup>1</sup>, EL GHADRAOUI Lahsen<sup>1</sup>, AL FIGUIGUI Jamila<sup>1</sup>& EL HARCHLI EL Hassan<sup>1\*</sup>

**Summary:** The sector of truck-farming horticulture knows numerous sanitary problems the most incriminated agents of which are mushrooms and bacteria'sleaves or roots. In Morocco, the lettuce *Lactuca sativa* is one of the most cultivated legumes. However, it's affected by several pathogenic agents among whom *Bremia lactucae* Regel, a mushroom responsible for the mildew or miller of the lettuce. The chemical fight remains the most used against this problem in spite of the Man and environment fatal effects. The present work concerns the study of certain *Lactuca sativa* biological parameters, under the influence of two chemicals: azoxytrobine and mancozèbe, widely used by Moroccan farmers, and also *Rosmarinus officinalis* essential oil. To do it, we led a series of experiments to the ground, in an agricultural plot of land situated to "Ain Beida" in Fez region. The studied parameters are: the number of leaves, the foliar surface, the rate of fresh and dry material and the chlorophyll rate. The obtained results have on one hand, shown an important biomass of *Lactuca sativa* handled by the rosemary essential oil, compared with untreated witnesses plants; on the other hand, we noted a positive effect of this vegetable essence which increases with the used concentration. However, the treatment made by azoxytrobine revealed a lesser effects of the plants physiology with regard to the treatment by mancozèbe. In addition, the processing of *Lactuca sativa* with the essential oil gave an important rate of dry air part material.

**Index terms:** *Lactuca sativa* L., essential Oil, *Rosmarinus officinalis* L., Mildew, azoxytrobine, mancozèbe

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## I. Introduction

The culture of the lettuce covers a surface about 880 000ha at the world level; with a commercial production which reaches 20 million tons. In Morocco, it occupies 1200 ha [1], the dominant variety being the sucrine lettuce. *Lactuca sativa* is one of the legumes the most sensitive to numerous cryptogamic, viral, bacterial diseases and to attacks by devastating. The most dreaded mushrooms are: *Botrytis cinerea* responsible for the grey decay; *Rhizoctonia solani* agent causing the decay of leaves; *Sclerotinia minor* responsible for the decay of the snare and for the basis of leaves; *Bremialactucae* Regel (on 1843) main pathogenic agent of the mildew or miller of lettuce; this one, be the object of the present study. It develops during all the period of the culture, as well outdoor, when under shelter and practically, all year round. This situation is due to the recurring bypassing of the genetic resistances by new origins of *Bremia* and to the weakness of the possible biological methods against this mushroom [2]. To face the renowned attacks of these bio-aggressors, the chemical fight remains the most used by the farmers. Unfortunately, this one presents fatal effects for the Man and environment. At present, the tendency towards the alternative solutions is more and more increased. It includes the integrated fight the biopesticides and the cultural best practice which take into account psychosocial conditions of the farmers, as well as pedo-climatic conditions of the considered zones. However, these are in phase of experiment or in very limited uses.

(<sup>1</sup>)Laboratoy of Functional Ecology and Environment, Faculty of Science and Techniques. Sidi Mohammed Ben Abdellah university, Road of Immouzer. P. B. 2202.

Fez-Morocco

\*Corresponding author: Elharchli Elhassan  
[hassanelharchli@gmail.com](mailto:hassanelharchli@gmail.com)

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In the present work, we tested two chemicals widely used by the farmers in the region of study (Dithane M45 and Ortiva 25cc). At the same time, a treatment with aromatic and medicinal plant (*Rosmarinus officinalis*) was led in the field, to know if this one could be an alternate way instead of the chemical processing.

Both used chemicals are described in the phytosanitary Index of Morocco as widely used fungicides [3],[4]. Ortiva 25cc is characterized by its systematic function with active ingredient: the azoxytrobine (250g/l), approved for the preventive and curative treatment of the diseases of lettuces, especially the mildew. Dithane M45 is a fungicide of contact having the mancozèbe, in 80 % content as active ingredient, authorized in the sale, used by the farmers of truck farming, although is approved on the culture of lettuce [4]. Both fungicides: Ortiva 25cc and Dithane M45 are widely used in Morocco to fight against several diseases, as the fleck, the alternariose and the anthracnose. *Rosmarinus officinalis* is an aromatic and medicinal plant widely spread in Morocco [5], its essential oil is used as preservative agent in foodstuffs to protect them against any microbial or fungal deterioration. These have a strong activity on the filamentous mushrooms and the protozoons [5]. Although numerous recent studies

showed that essential oil and their constituents present an important potential as antimicrobial agents in several industrial and medical domains. The works led on their efficiency against the diseases of the lettuce remain very limited even absentees, where from the interest of the present study the experiment of which was applied, in full field (natural conditions).

## II.MATERIEL AND METHODS

### 2.1 Plant material

*Rosmarinus officinalis* is a botanical species having a wide distribution. it is met in the Saharan Atlas, the High Atlas, Middle Atlas (abounding in the valley of Skoura-Mdez and on lapels of the oriental Middle Atlas), Rif and in the mountains of oriental Morocco (abounding on BeniSnassène and chains of Debdou-Jerada) [6]. The variety of tested lettuce is fat sucrine lettuce of the species *Lactuca sativa* leaves of which are darkened and thick, having a sweet flavor and characterized by a small crinkled firm apple.

### 2.2 Conditions of culture

The experiment was realized on an agricultural plot of 24m<sup>2</sup>. The analyses of the substratum were previously made and showed that the pedological parameters in the region of study answer the standards culture of the truckfarming products. The sowing of the culture took place at the beginning of October (year 2014), and the harvest was made during next February. One month after the culture in tree nursery, seedlings are transplanted in a trial plot of land according to a complete random block. Six used lines have a length of 4meter and spaced out by 80 centimeter. Is thirty lettuce seedlings are transplanted at the rate of two ranks by line and spaced out of 25centimeter to allow a good aeration (Fig. 1). We so have, a line untreated witness, lines having sudden a treatment in the oil of the rosemary with various doses and lines handled by mancozèbe and/or azoxytrobine with the approved dose.



Figure 1: experimental site of study

The watering of the culture is made by micro-irrigation, known under the name of "system of drip" with an average debit (2,5liter / hour). The organic fertilization of the ground is brought one month before the sowing, in the form of fertilizer (rich in essential elements) at the rate of 10t / ha,. The mineral fertilization brought to the exploited plots of land is constituted by main elements N: nitrogen, P: phosphate; K: potassium (Table 1).

Table 1: Type of fertilizers brought to the lettuce culture

Type of fertilizer	Dates contribution	Formula of the dosage
P <sub>2</sub> O <sub>5</sub> + N	15 daysaftertransplanting	The ratio N-P-K : 3-1-3
K <sub>2</sub> O	20daysaftertransplanting	
N + K <sub>2</sub> O	35daysaftertransplanting	

### 2.3 Applied treatments

Four types of treatment are used:

- Traitement in Dithane M45 (P1) with the mancozèbe (80 %), very wide-spread used agricultural brought in statutory dose.
- Treatment in Ortiva 25cc (P2) with active molecule (azoxytrobine 25g/l), belonging to strobilurnes, (family which marked the beginning of a new era) [7].
- Treatment associating both fungicides (P1+P2).
- Treatment with vegetable essences of *Rosmarinus officinalis* in two different concentrations.

All the treatments are applied, after transplanting, under the shape of liquids sprayed on plants, at the rate of an application by 15 days.

During the cycle of culture, we established a periodic follow-up, throughout the various stages of lettuce development. This, allowed us to highlight the possible appearance in time, of such or such disease or of attack by devastating. Three months after the sowing, plants were collected and four parameters were studied: the biomass in the fresh state and in the dry state, the foliar surface and the rate of chlorophyll.

► **Biomass:** the fresh underground and air parts of every foot of salad (sheets, stalk, pivot, roots) were weighed and their weight is estimated after drying in steam room in 72 hours in 70°C [8].

► **Rate of chlorophyll:** the dosage of the chlorophyll was made by using the method of Hiscox and Israelstam (1979). This one consists in putting 30 - 40 mg of a fresh leaf of every plant in 4ml of DMSO. Tubes so got back are put in the steam room in a temperature of 65°C during 15min and then the optical density was measured in two wavelengths 663nm and 645nm, The calculation of the rate of the total chlorophyll was made according to the following formula [9].

$$\text{Total Chl( g/l )} = 0,0202 * A645 + 0,00802 * A663$$

► **Foliar surface:** the collected leaves are weighed and are then, scanned in the form of image and printed in size A4, then cut according to their dimensions to calculate the foliar surface.

#### 2.4 Statistical test

The evaluation of the products effect used on the lettuce culture was realized by the analysis of the variance in a single factor (ANOVA) of each of the variables studied: fresh Biomass and sandbanks, rate chlorophyll and foliar surface.

For the results which are in the form of percentage, we used a transformation of data to meet the conditions of normality of the distributions and the equality of the variances. This angular transformation was realized according to the following formula:  $= 2 \text{ArcSin} x / 100$  [10]. The statistical analysis realized by the program SYSTAT version 12.

### III. RESULTS AND DISCUSSION

The results obtained are represented in the table below.

**Table 2: devastating and diseases observed on the lettuce during its cycle of culture**

Period	Stage of culture	Observations	Symptoms
End of tree nursery - beginning of transplanting	6 leaves	Mildew of lettuce	Green Tasks on superior face of leaf and grey felting on lower face.
10 days after transplanting	8-9 leaves	Presence of aphids" <i>Nasonoviaribisnigri</i> "	aphids at level of apple and inside leaves
20 days after transplanting	10 leaves	Obstnacy of mildew on witness	Obstnacy of symptoms
One month after transplanting	12-13 leaves	Disappearance of mildew on witness	-
45 days after transplanting	14 leaves	Appearance of alternariose	Brown Tasks bounded well by different size on leaves.

The diseases observed on the lettuce, during its cycle of culture, are easily recognizable. In our case, the symptoms of the mildew appeared early enough. The lack of aeration and the ruling wet conditions in the region in this period of study are supposed by the main factors favoring the appearance of the mildew. The latter is considered as the most dreaded disease for

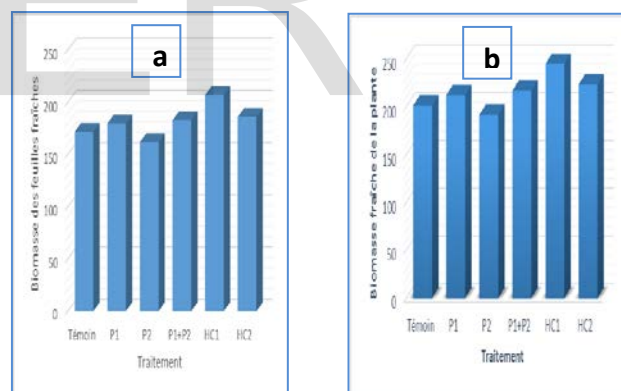
this species, in particular in biological winter culture [11]. The mushroom can develop in any stage of culture.

In our experiment, the trial period is considered the most pluvial of the year. The conditions of stress such as, ice-cold and low luminosity could explain the sensibility of the lettuce to the mushroom in question. The various chemical or biological treatments brought from the appearance of the disease, were able to eliminate the germ, knowing that the latter persisted on the witness (untreated).

The disappearance not expected from the disease on the witness justifies itself probably, by the routing of chemicals, used by the farmer, by the roots of witness'plants, or through the scattering of these substances by the wind during the pulverizing of the neighboring plots of land.

#### ► Fresh biomass

Graphical representations (a) and (b) show the development of the leaves biomass and whole plant to the fresh state according to the various applied treatments. It emerges that the treatment by rosemary essential oil used at the rate of 2 gout by water liter, allows obtaining the best biomass. On the other hand, treatments by chemicals show no effect on the biomass in comparison to the witness.

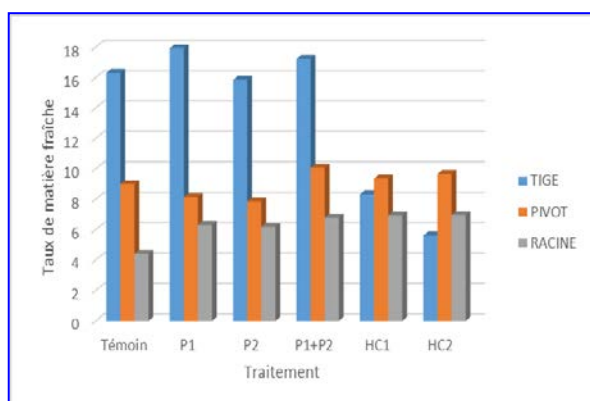


**Figure 2: effect of the various treatments used on the biomass of the fresh leaves ( a ) and the whole plant ( b ). P1: fungicide with Mancozèbe 80 %; P2: fungicide with Aoxystrobine 250 g / liter; HC1: rosemary essential oil diluted (2 gout by liter); HC2: rosemary essential oil diluted (4 gout by liter).**

As for the biomasses of the stalk, the pivot and the root (figure 3), we were able to show that the best biomass of stalks is observed with the use of chemicals P1 and P1+P2. On the other hand, the biomass of roots and pivots shows itself more important with the use of essential oil.

The effect observed in the treatment of P1+P2, can be allocated to P1, seen that the azoxytrobine (produced P2) does generally not present difference with regard to

the witness. On the other hand, previous studies showed that the same substance produces 8 in 10 % of additional biomass in the wheat leaves [12].



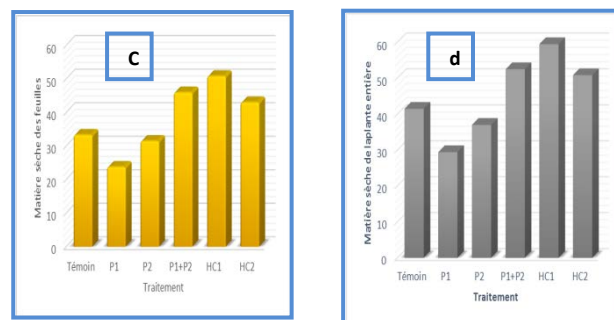
**Figure 3: rate of fresh material (stalk, root and pivot) according to the used treatments. P1: fungicide with Mancozèbe 80 %; P2: fungicide with Azoxystrobine 250 g / liter; HC1: rosemary essential oil diluted (2 goutts by liter); HC2: rosemary essential oil diluted (4 goutts by liter)**

The obtained results show that rosemary essential oil presents a beneficial effect on the various studied biological parameters, except the stalk and the pivot roots. Also, the association of two products (P1+P2) showed a similar effect on the biomass of the whole plant and on the various parts of lettuce. The treatment of the same culture by several pesticides is an irrational use, which could have of negative consequences. So, the pulverizing of active substances and their metabolites entail impacts on the various compartments of the environment (ground, air, water). The latter can persist there or be degraded according to the active material and the nature of the ground [13] .

#### ► Biomass dries

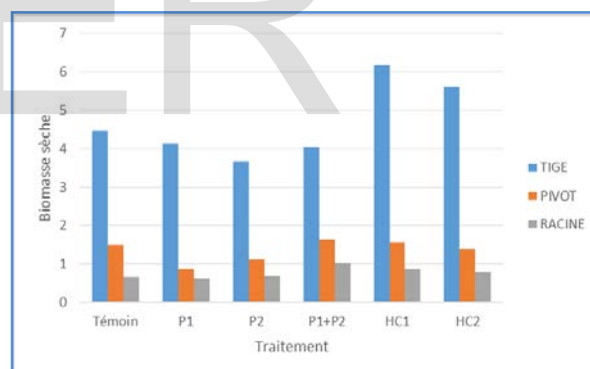
Figures (c) and (d) below, illustrate the rate of dry material of lettuces cultivated according to the type of brought treatment. The obtained results show themselves in accordance with those of the fresh leaves biomass and whole plant. The good biomass is noted at salads handled by Rosmarinus essential oil, used at the rate of 2 goutts by liter of water. And in a parallel to the data deducted from the fresh material, it is the treatment by the combined of both fungicides that marked a result comparable to the biological treatments. The graphical representations of the results showed us that the effect of the azoxytrobine is negative. The values so obtained are relatively similar to those of the witness. Of these results, we can underline that P2 shows no effect that in case of combination with P1. Rosemary Essential oil and the treatment combined both fungicides reveal a

positive effect on the dry biomass of the whole plant. This impact is less considerable at the dry leaves biomass and even less for the pivot and the roots.



**Figure 4: rate of material dries leaves (c) and whole plant (d) according to the brought treatment [P1: Fungicide with Mancozèbe 80 %; P2: fungicide with Azoxystrobine 250 g / liter; HC1: rosemary essential oil diluted (2 goutts by liter); HC2: rosemary essential oil diluted (4 goutts by liter)]**

Concerning the biomass dry other parts of plant (stalk, pivot and roots), it presents peak to lettuces handled by essential used oil HC1 (figure 5), followed by that handled by the combined (P1+P2), this is in accordance with the results of the dry biomass in whole plant, but disaccord with the results of the fresh biomass of the stalk.



**Figure 5: rate of the biomass dries (Stalk, root and pivot) according to the used treatment [P1: Fungicide with Mancozèbe 80 %; P2: fungicide with Azoxystrobine 250 g / liter; HC1: rosemary essential oil diluted (2 goutts by liter); HC2: rosemary essential oil diluted (4 goutts by liter)]**

*Rosmarinus officinalis* essential oil seems endowed with an antimicrobial activity which depends mainly on their chemical composition [14]. Our results allow supposing that these vegetable essences used in strong concentration (HC2) show a very beneficial effect on the biomass of *Lactuca sativa*.

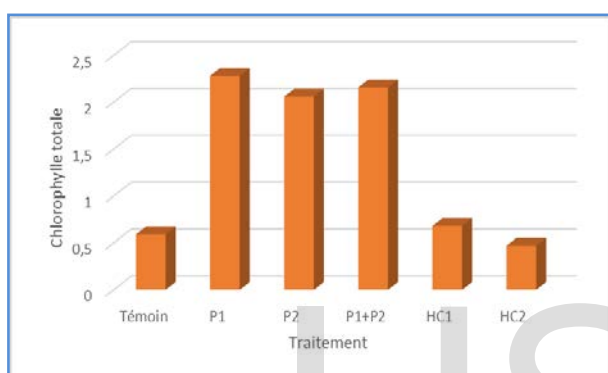
The Product P1 (Dithane M45) with Mancozèbe, showed a positive effect relatively identical to that recorded by the treatment HC1. These results show themselves comparable with those brought reported by [2] in an experiment of fight against *Bremia lactucae* by



using a fungal product with copper and having obtained a notable effect on the biomass of the handled salads. On the other hand the product P2 (Ortiva 25) with Azoxystrobine shows no effect on the studied parameters and this in comparison with the witness.

► **Rate of chlorophyll**

The dosage of the chlorophyll has and b and the total chlorophyll, revealed that the photosynthetic reaction takes place in a more intense way at plants handled by pesticides, compared with the witness. On the other hand, essential oil used at the rate of 4gouttes / liter entailed a decrease of the chlorophyll rate with regard to the witness (Figure 6).

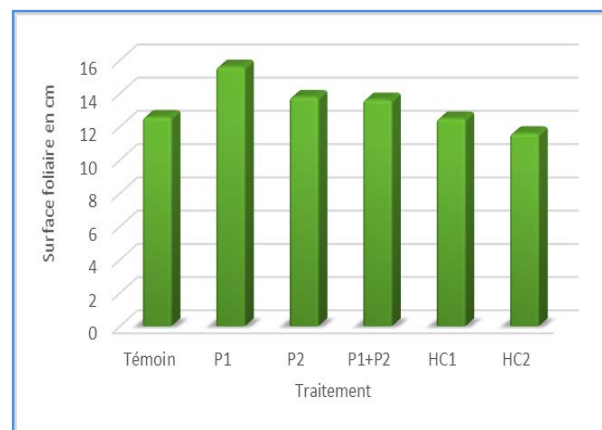


**Figure 6: rate of total chlorophyll according to treatments [P1: fungicide with Mancozèbe 80 %; P2: fungicide with Azoxystrobine 250 g / liter; HC1: rosemary essential oil diluted (2 goutts by liter); HC2: rosemary essential oil diluted (4 goutts by liter).]**

The azoxystrobine being endowed with systematic properties penetrates inside tissues of the host to arrest the development of mushrooms and block so the progress of the disease [12]. In our study, it was able to develop considerably the content in chlorophyll in the fresh leaves. This can be explained by the foliage of lettuce leaves which are maintained green and functional longer, so assuring a long period of photosynthesis. As this was brought reported in a study on the wheat [15].

► **Foliar surface**

The results obtained on the foliar surface of the fresh lettuces according to treatments are given in the figure7. These show that this one, is slightly important at plants treated with the mancozèbeas a contact fungicide which applies and reacts to the superficial level.



**Figure 7: variations of the foliar surface of the fresh leaves according to treatments [P1: fungicide with Mancozèbe 80 %; P2: fungicide with Azoxystrobine 250 g / liter; HC1: rosemary essential oil diluted (2 goutts by liter); HC2: rosemary essential oil diluted (4 goutts by liter).]**

**IV.CONCLUSION**

The various treatments brought in our experiment of the field, allowed observing a notable effect of rosemary essential oil on the parameters bound to the fresh and drybiomass of lettuces and it is true in comparison with the mancozèbe and the azoxytrobine. Plants treated with this essential oil knew a considerable evolution at the level of the dry air partmaterial in plant, but less important on the contents in total chlorophyll and foliar surface. The evolutions, so noted, are more remarkable at salads handled by the concentration lower (HC1). Also, the combination of both fungicides influenced positively the growth of the salad by a development of root and air part, but we suppose that it, is essentially due, in the product P1 with the mancozèbe, because the results obtained by the use of the combined are very moved closer to those of the treatment by Dithane M45. The treatment by *Rosmarinus officinalis*essential oil could be an effective alternate solution to the chemical fight in truck-farming production, as long at the level of the yield, that of the product quality. The biological fight by this essential oil on the mildew is a point which deserves to be developed, by taking into account several other factors.

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