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In Vitro and *In Vivo* Effects of Aqueous Extract of *Rosmarinus officinalis* L. (Rosemary) in The Control of Late Blight Disease of Potato Caused by Phytophthora Infestans Mont. De Bary. in Algeria

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Abstract

The fungus *Phytophthora infestans* is known to develop resistance against the metalaxyl (fungicide), commonly used in the control of potato mildew disease. There is therefore urgent need to explore the potentials of alternative fungicides which are potent, affordable, readily available, easy to prepare and environment friendly. The study was carried out to test the fungicidal potential of aqueous extracts of *Rosmarinusofficinalis* (Rosemary), *in vitro* and *in vivo* on two isolates of *P. infestans* collected from two potato producing Algerian areas: Bourkika (Tipaza City) and El Abbadia (Aindefla City). Various concentrations of crude extracts of *Rosmarinus officinalis* applied by direct contact in the following dilutions: 5%, 10% and 20% on medium with pea-agar (PPA), allowed the inhibition of mycelial growth of *P. infestans* isolates. The observed rates of inhibition exceeded 85% and the inhibitive minimal concentration (CMI) was 5%. Parallel structural modifications, caused by mycelial lyses, as well as the deformation or, and the digestion of the contents of sporangia affected the morphology of both strains from the lowest concentration. The sporulation and the germination were inhibited by this aqueous extract (100%). Also, the absence of resumption of mycelial growth on medium PPA and absence of the mildew symptoms on detached Spunta potato leaves confirmed the fungicidal effect of the Rosemary aqueous extract. This also translated *in vivo* as significant reduction of the disease was observed. Disease reduction was recorded for the preventive application modes by spraying with the crude aqueous extract (86.2%) and by watering, while for the curative mode with crude extract (81%). On the other hand, Spunta variety was more marked for preventive mode by watering (85%) and the curative one (90%) also, A2 isolate was more inhibited for the application of *R. officinalis* aqueous extract by curative (83%), spraying mode (86%) and watering modes. Besides, treatments made in preventive modes by spraying and watering showed a total inhibition of the sporulation (100%), exceeding 85% in Spunta variety and 96% for A1 isolate was observed in the curative mode of application. This study thus confirms the antifungal potential of aqueous extract of *Rosmarinus officinalis* on *P. infestans* isolates. It is thus recommended for use as bio-fungicide in the management of potato mildew disease.

Keywords: *Rosmarinus officinalis*; *Phytophthora infestan*; Fungicide; *Solanum tuberosum*

Introduction

Late blight potato, caused by *Phytophthora infestans* is one of the most destructive diseases of potato. Until now, chemical control remains the most important control against the disease. However, the use of pesticides has several constraints such as high cost of fungicides, negative effects on the environment and the health of the consumers [1]. Also, the appearance of aggressive isolates of this fungus, mostly resistant to the current synthetic fungicides, has created new challenges for potato growers [1].

Various reports have highlighted the action of certain plant extracts and some essential oils against the phyto pathogenic agent of potato mildew disease [2].The experiment, therefore aimed at evaluation of the antifungal activities of crude aqueous extract of *Rosmarinus officinalis* on *Phytophthora infestans* isolates, while determining *in vitro* the effect of the extract on the mycelial growth, sporulation and germination, the determination of the minimal and lethal inhibitory concentrations, as well as the inhibition of their survivability after treatments. Also, observations were made *in vivo* on detached potato's leaves, its effect on the symptoms appearance period, disease reduction and sporulation inhibition.

Materials and Methods

Plant material: The plant material used includes the aerial parts composed of stalks, leaves and flowers of rosemary (*Rosmarinus officinalis* L.) as well as seed potato tubers. The collection of plants was made in May, 2011 in Medea city in M'sallah locality. After the harvest, the plant material was cleaned with tap water to clear it of fragments of soil, then it was left to dry away from direct sunlight, at ambient temperature and in open air.

Two approved varieties of potato, certified and widely cultivated in Algeria, Spunta and Kondor were collected and retained for *in vivo* study. Seed tubers were supplied by the National Center of Control and Certification of seeds and seedlings (C.N.C.C) of EL Harrach, Algeria.

Fungal isolates: Two purified fungal isolates of *Phytophthora*

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infestans, identified respectively as A1 and A2, were selected for this study. The latter was taken from potato producing areas: El abadia of Ain defla city and Bourkika of Tipaza city. They were maintained by transplanting on pea- agar medium and incubated at 18°C during 20 days [3].

Preparation of rosemary aqueous extract: The aqueous extract was obtained by decoction of 100 g from dried plants in 1 L of distilled water and heated in the autoclave at 100°C for 30 minutes in well closed vials to avoid contamination.

The extract was filtered using Whatman's sterile filter paper in the laboratory. The obtained filtrate was collected in sterile glass vials hermetically closed and stored in a refrigerator at 4°C until its use in the following dilutions: 5, 10, 20 and 100% [4,5].

Potato varieties cultivation: Pre-germinated potato tubers were planted in pots previously prepared (at a rate of one tuber per pot) and depth of 4 to 5 cm, the substrate constituted a mixture of 2/3 of unused soil and 1/3 of peat [6].

Planting was done in 12 pots among which 6 were reserved as controls. The planting was replicated thrice for each variety. Aqueous extract were applied to the soil in 6 pots, to field capacity, every three days at a dilution of 20% from date of planting to pre-flowering. The control experiment was irrigated with clean tap water.

***In vitro* study:** This part of study was based on inhibition of mycelial growth, sporulation and germination of both isolates of *P. infestans.*

Mycelial growth inhibition: Four concentrations: 5%, 10%, 20% and 100% of *R. officinalis* aqueous extract were treatments used for this study, and correspond respectively to treatments D1, D2, D3 and D4.Mycelial growth inhibition was based on direct contact method, described by Mishra and Dubey [7].The microbiological procedures and the minimum inhibitory concentration (MIC) of the aqueous extract were determined according to Paranagama et al. [8] method For each treatment, 5 ml of plant extract was poured into Petri dishes of the same diameter (90 mm) using micropipettes. The Pea- agar medium was maintained in surfusion (45°C) then poured into Petri dishes containing aqueous extract. The latter were slightly shaken to homogenize the medium. Plant aqueous extract in the control experiment was substituted by sterile distilled water. Treatments were replicated five times for each *P. infestans* isolate.

Using sterile Pasteur pipettes, a disk of 50 mm in diameter of inoculum, for each isolate was taken and inoculated at the center of Petri dishes. Incubation of plated dishes in the hot air oven was done at a temperature of 18°C to evaluate the mycelial growth, which was daily observed for a period of 15 days. Readings were taken by calculating the average of two diameters measured on two perpendicular axes drawn on the reverse side of the plated petri dishes.

The minimum inhibitory concentration of mycelial growth (MIC) was determined for each isolate.

Mycelial growth inhibition rate was determined for each *P. infestans* isolate according to the formula described by Rollan et al. in Ibarra-Medina et al. [9].

$$I(\%)=\frac{(DT-Dt)}{DT}\times 100$$

Where I is a percentage Inhibition rate of mycelial growth of *P. infestans* isolate,

DT is a Mycelial growth (mm) of *P. infestans* isolates in control and

Dt is a Mycelial growth (mm) of *P. infestans* isolates developed in the medium in the presence of *R. officinalis* aqueous extract.

Antifungal activity of rosemary aqueous extract on morphology of *P. infestans* isolates: In evaluating the effects of rosemary aqueous extract on the phyto pathogenic isolates, a morphological description was done after 15 days of incubation, by direct observation of the treated cultures and controls of A1 and A2 of *P. infestans* isolates under photonic microscope at magnification (X125).

Sporulation and germination inhibition: After incubation for 21 days, at 18°C, each plated petri dish was collected, and 15 ml of sterile distilled water poured in, and then scraped with sterile Pasteur pipette to recover separately sporangial suspensions in sterilized test tubes. These were agitated using an agitator of tubes vortex. The sporangial suspensions prepared for A1 and A2 isolates were observed to determine the concentration of spores using a hemacytometer under optical microscope. Five repetitions were carried out for each fungal isolate, and each concentration to calculate sporulation inhibition rates according to the formula of Hibar et al. [10].

$$IS(\%)=\frac{(ST-St)}{ST}\times 100$$

Where IS is a percentage Inhibition rate of *P. infestans* sporulation,

ST is a concentration in sporangia of control *P. infestans* isolates (sporangia.ml^{-1}) and

St is a concentration in sporangia of *P. infestans* isolates developed in the medium in the presence of *R. officinalis* aqueous extract (sporangia. ml^{-1}).

Parallel, germination inhibition rate (IG%) was calculated for each isolate, according to the formula described by Hill and Nelson [11].

$$IG(\%)=\frac{(NT-NPA)}{NT}\times 100$$

Where IG is a percentage germination inhibition rate of *P. infestans* strain,

NT is a concentration of *P. infestans* sporangia germinated in control (sporangia.ml^{-1}) and

NPA is a concentration of *P. infestans* sporangia developed in medium in presence of *R. officinalis* aqueous extract (sporangia.ml^{-1}).

Survivability of treated *P. infestans* isolate: To evaluate the fungistatic and fungicidal effects of *R. officinalis* aqueous extract, *in vitro* and *in vivo* survivability of *P. infestans* isolates previously treated were monitored respectively on PPA medium and on leaf disks of Spunta potato's cultivar.

In vitro survivability study was based on the technique modified by Mahanta et al. [12].The test was based on the resumption or the absence of mycelial growth on the isolates inhibited by *R. officinalis* aqueous extract. Explants were transplanted on fresh Pea- agar medium under conditions of incubation previously mentioned. Four explants of each *P. infestans* isolate were transferred to Petri dishes, with four replicates. Treatments were administered and observations made in comparison with the control. Readings were taken daily, for 7 days.

The lethal inhibitory concentrations (CIL) were estimated at the end of the experiment. The CIL was determined from the smallest

concentration for which no mycelial growth and no resumption of the explant was observed on the PPA medium in the term of 7 days of incubation [8].

Besides, the *in vivo* survivability of *P. infestans* isolates beforehand treated with the plant extract at different concentrations was realized according to the method of Klarfeld et al. [13].

Healthy detached Spunta potato leaves having a diameter greater or equal to 50 mm were chosen and collected from healthy plants in Tipaza city. The leaves were cut to uniform disks using a punch, they were washed with clean tap water then disinfected in 2% of Sodium hypochlorite solution for 3 minutes, then rinsed in 3 changes of sterile distilled water.

Sterile filter paper moistened with sterile distilled water was deposited in transparent plastic and sterile boxes, a plastic mesh was also placed in, then 5 potato leaf disks were placed in the box, and the explants of isolates were introduced. Previously treated leaves along with the controls were also observed.

Incidence of the disease was defined by the number of leaf disks presenting typical symptoms of mildew, while disease severity was represented by expression of the symptoms in terms of percentage of surface infected by the mildew. Disease reduction rate was calculated using the formula proposed by Hill and Nelson [11].

$$\text{Inf}(\%)=\frac{(\text{InfT-Inft})}{\text{InfT}}\times 100$$

Where Inf is a percentage infection rate of detached potato leaf disks,

Inf T is a% infection rate of positive controls detached potato leaf disks and

Inf t is a% infection rate of detached potato leaf disks treated by aqueous extract.

***In vivo* antifungal potential of *R. officinalis* aqueous extract:** *In vivo* antifungal potential evaluation was done by the application of potato leaf disks with treatments *in vivo* as well as the leaf disks controls inoculated by A1 and A2 of *P. infestans* isolates. Various modes of treatment were used for this study:

- Preventive application through spraying potato leaf disks with rosemary aqueous extract at concentration of 20% for few minutes. 24 hours after the treatment, 100 µl of sporangial suspension of 10^5 sporangia.ml^{-1} were deposited by means of a micropipette on the lower surface of potato leaf disk at 5 replications per fungal isolate.

- Curative application through the inoculation of potato leaf disks by depositing 100 µl of sporangial suspension on the lower leaf surface, then after 24 hours, application of droplets of 50 µl crude aqueous extract of *R. officinalis* at 20% concentration.

- Disks of detached potato leaves earlier treated with *R. officinalis* aqueous extract diluted at 20% were inoculated with 100 µl of sporangial suspension of *P. infestans* and incubated at 18°C for 10 days in the sterile transparent boxes.

The frequency of attacks was estimated two to four days later.

Both negative and positive controls were observed. Negative control, in which the disks of detached potato leaves were treated with sterile distilled water, and positive controls, where the detached leaf discs were inoculated with A1 and A2 of *P. infestans* isolates [14,15]. *In vivo* antifungal potential evaluation of *R. officinalis* aqueous extract on *P. infestans* isolates was done using the following parameters.

Period of appearance of the symptoms: It is the necessary time for the appearance of the infection by the phyto pathogenic agent on the inoculated foliar tissue.

Reduction of late blight disease: The reduction of the disease or (%DR) was translated by the product of the incidence of the disease (number of infected leaf disks) by the scale attributed to the infected foliar surface. It is determined by the formula proposed by Hill and Nelson [11].

RM (%)= CIP- CIPE/ CIP x 100

Where CIP is a coefficient of infection of controls (detached potato leaves inoculated with *P. infestans* isolates.),

CIPE is a coefficient of infection of treated detached potato leaves inoculated with the phyto pathogenic isolates.

Inhibition of the sporulation: After 10 days of incubation, the infected disks of detached potato leaves were carefully dipped into sterile tubes containing 10 ml of sterile distilled water then subjected to agitation by means of an agitator of tubes vortex to release the sporangia produced. The content of each tube was observed to determine the concentration of spores by means of a Hemacytometer under optical microscope at magnification (X125).

Sporangial production inhibition rate or IPC was calculated using the formula proposed by Hill and Nelson [11].

IPC (%)= NCP- NCPE/ NCP x 100

Where NCP is a number of sporangia produced on surface of detached potato's leaf disk inoculated by *P. infestans* isolates,

NCPE is a number of sporangia produced on surface of detached potato leaf disk treated with *R. officinalis* aqueous extract and inoculated with *P. infestans* isolates.

Statistical analysis: Data obtained was analyzed using Analysis of variance, ANOVA SYSTAT vers.7, variance calculated using the GLM (Generalized Linear Model), the differences were considered significant for $P < 0.05$.

Results and Discussion

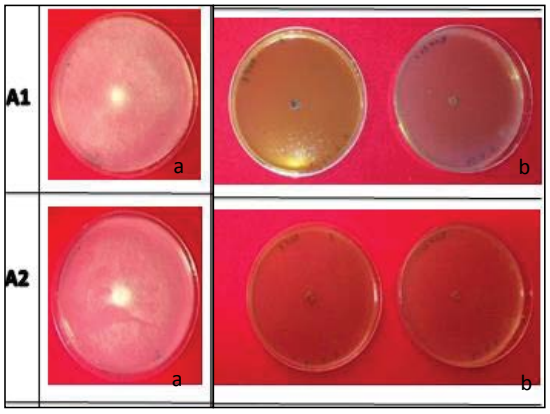
In vitro antifungal potential

Evaluation of mycelial growth inhibition: The analysis of variance of mycelial growth inhibition showed statistically significant differences between the two isolates, but no significant difference between the various *R. officinalis* aqueous extract studied at different concentrations (Table 1). In GLM, the latter exceeded 85% for 5% concentration to evolve slightly to 20% concentration where, it was more pronounced on A1 isolate. Therefore, 5% concentration represents the minimum inhibitory concentration (CMI) of *R. officinalis* aqueous extract (Figures 1 and 2).

Antifungal effects of *R. officinalis* aqueous extract on *P. infestans* isolates: The inhibition of the mycelial growth of *P. infestans* isolate could have resulted from the effect of the extract causing lysis and vesiculation of the mycelium, as well as the deformation of sporangium and the digestion of their contents. These morphological modifications were also observed from the lowest concentration of this tested plant extract (Figure 3).

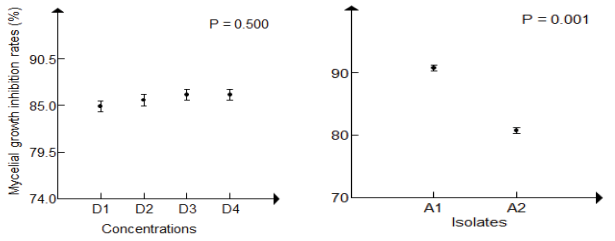
Factors	Sum-of-Squares	ddl	Mean- Square	F-ratio	P
Concentrations	2.635	3	0.878	1.000	0.500
Isolates	200.983	1	200.983	228.812	0.001

Table 1: Variance analysis of *P. infestans* mycelial growth inhibition rates according to rosemary aqueous extract concentrations and isolates.



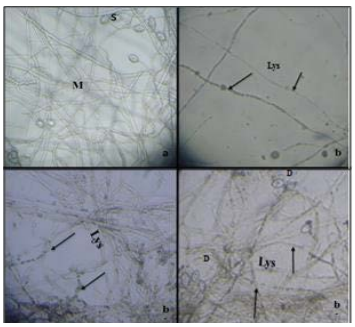
a: Controls strains
b: Treated strains respectively at 10 % and 20 %.

Figure 1: Mycelial growth variability of A1 and A2 *P. infestans* isolates under treatments of rosemary aqueous extract at different concentrations.



D1, D2, D3 and D4: Concentrations respectively at 5%, 10%, 20% and 100%

Figure 2: Variance analysis of mycelial growth inhibition rates of *P. infestans* in GLM according to rosemary aqueous extract concentrations and isolates.



M: Mycelium; S: Sporangia; Lys: Lysis of Mycelium; D: Digest of content of sporangia; a:Control isolate; b:Treated isolate

Figure 3: Morphology of controls (a) and treated (b) isolates of *P. infestans* by rosemary aqueous extract

Sporulation and germination inhibition of *P. infestans* isolates: The sporulation, as well as the germination of the studied isolates were affected by aqueous extract of *R. officinalis* at 5%, concentration, where 100% inhibition was observed.

Effect of Rosemary aqueous extract on the survivability of *P. infestans* isolates: Concerning the *in vitro* study, the isolate A1 was more sensitive to the treatment and, the inhibition evolved with aqueous extract tested at different concentrations. Also, inhibition was observed *in vivo* for both isolates and four concentrations of the *R. officinalis* aqueous extract. However, the lethal inhibitory concentration (CIL) of the isolate A1 was higher than that of the A2 isolate.

In vivo antifungal potential

Determination of the symptoms appearance period: The analysis of variance of the symptoms appearance period did not show significant differences between the treatments application rates, potato varieties, *P. infestans* isolates and *R. officinalis* aqueous extract (Table 2).

Variability in the period of symptoms appearance was observed among the treatments. It was more in crude than in diluted extracts. However, it was approximately similar for both concentrations of aqueous extract applied with respect to the curative rate (2.6 days).

On the other hand, the periods of symptoms appearance by activity of both isolates of *P. infestans* showed that A1 isolate reproduced the symptoms more quickly than A2 isolate for various treatment application rates.

The symptoms appearance period extended till the 3rd day. The shortest period was marked for the preventive mode by watering while the mode of spraying with the crude aqueous extract showed the longest period. Therefore, the classification of the various application rates was established in the following decreasing order: preventive by spraying with the crude extract (SPR1: 3 days) and, in diluted extract to 20% (SPR2: 2.8 days), curative by use of crude and diluted extracts (CUR1, CUR2: 2.7 days), preventive by watering (WAT: 2.6 days) (Figure 4).

Antifungal potential of rosemary aqueous extract on disease reduction: The analysis of variance of disease reduction rates did not show significant difference between the application rates, on both isolates of *P. infestans*, concentrations of rosemary aqueous extract applied for all application rates and potato varieties for the preventive mode by spraying and watering (Table 3).

However, a significant difference was noticed between potato's varieties according to the curative application mode (F=10.662, P=0.031) (Table 3).

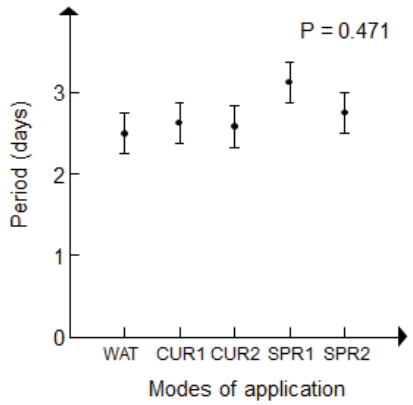
In GLM, the highest disease reduction was observed for the preventive mode in treatment with the crude extract (SPR1: 86.2%), while the lowest rate was observed for the preventive treatment by watering and curative with crude extract (WAT, CUR1: 81%).

Therefore, the classification of application rates was established in decreasing order.

- Preventive mode by spraying potato leaf disks with crude

Parameters	Application mode of treatments	Sumof squares	d.d.l	Mean square	F- Ratio	P
Modes of Application	All the modes	0.964	4	0.241	0.933	0.471
Varieties	Spraying	0.521	1	0.521	1.000	0.351
	Watering	0.125	1	0.125	1.000	0.374
	Curative	0.058	1	0.058	0.378	0.558
Phytophthora Infestans Isolates	Spraying	0.021	1	0.021	0.040	0.847
	Watering	0.125	1	0.125	1.000	0.374
	Curative	0.058	1	0.058	0.378	0.558
Concentrations	Spraying	2.882	2	1.441	2.766	0.130
	Curative	1.145	2	0.572	3.738	0.079

Table 2: Variance analysis of symptoms appearance periods of *P. infestans* according to the treatments application modes, concentrations, Potato's varieties and isolates



WAT: Watering by the crude extract SPR1: spraying with crude extract SPR2: spraying with diluted extract at 20% CUR1: curative treatment with crude extract CUR2: curative treatment with diluted extract at 20%.

Figure 4: Variance analysis of symptoms appearance period in GLM according to treatments application modes and concentrations.

Parameters	Application modes of treatments	Sum of squares	ddl	Mean square	F-Ratio	P
Modes of Application	All the modes	90.322	4	22.580	0.141	0.964
Varieties	Spraying	39.739	1	39.739	0.118	0.749
	Watering	52.345	1	52.345	5.395	0.259
	Curative	534.903	1	534.903	10.662	0.031
P. infestans isolates	Spraying	17.731	1	17.731	0.053	0.830
	Watering	22.515	1	22.515	2.320	0.370
	Curative	46.321	1	46.321	0.923	0.391
Concentra tions	Spraying	6.643	1	6.643	0.020	0.895
	Curative	0.574	1	0.574	0.011	0.920

Table 3: Variance analysis of disease reduction rates according to treatments application modes, rosemary aqueous extract concentrations, potato's varieties and *P. infestans* isolates.

aqueous extract (SPR1: 86.2%)

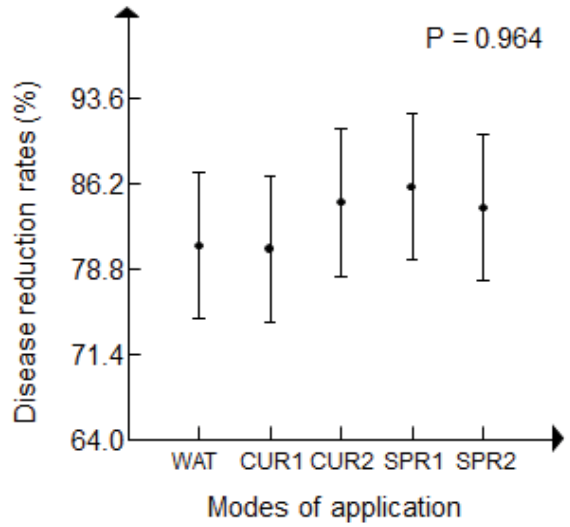
- Preventive mode by spraying potato leaf disks with diluted aqueous extract at 20% and curative mode with diluted aqueous extract at 20% (SPR2, CUR2: 84%)

- Preventive mode of watering and curative mode with crude rosemary aqueous extract (WAT, CUR1: 81%).

On the other hand, the disease reduction rates registered for all treatment application modes were (up to 70%) on both varieties, but the Spunta variety showed a more significant disease reduction for preventive mode treatment by watering (85%) and curative mode (90%) while it was more marked on kondor variety for the preventive mode by spraying (88%) (Figure 5).

Besides, it was important also for both isolates of *P. infestans* exceeding 75% for the various treatment application rates. On the other hand, the reduction affected much more A2 isolate for treatment application rates by spraying (86%), watering and curative (83%) (Figure 5).

This also confirms that the reduction of the disease was slightly



WAT: Watering by the extract SPR1: spraying with crude extract SPR2: spraying with diluted extract CUR1: curative treatment with crude extract

CUR2: curative treatment with diluted extract.

Figure 5: Variance analysis of disease reduction rates in GLM according to treatments application modes and concentrations.

higher in application of the crude treatments than with treatments diluted at 20% for both modes of application: preventive by spraying and curative.

Antifungal potential of *R. officinalis* aqueous extract on sporulation inhibition of *P. infestans*: The variance analysis of sporulation inhibition rates did not show significant difference between treatment application modes, potato's varieties, *P. infestans* isolates and, *R. officinalis* aqueous extract concentrations (Table 4).

In GLM, all the sporulation inhibition rates registered showed antifungal effect against *P. infestans* sporulation (rate exceeding 75% and reaching 100%) (Figure 6).

- Classification of treatments application rates was established in the following decreasing order.

- Watering by the crude aqueous extract and spraying with crude and diluted at 20% of rosemary aqueous extract (100%).

- Curative mode with a crude and diluted at (20%) of rosemary aqueous extract (77%).

The inhibition of sporulation was recorded on both varieties in curative application. Spunta variety showed higher inhibition (over 85%) than Kondor (bordering 55%).

The latter was variable on both isolates of *Phytophtora infestans*. Sporulation of A1 isolate was higher than A2 isolate for curative mode (over 96%), while the rate registered for A2 isolate borders 45%.

On the other hand, a slight variation of sporulation inhibition rates was noticed between both concentrations of aqueous extract applied for the curative mode (77%) for the crude extract and (68%) for the diluted extract at concentration of 20%.

It is very important to indicate that the treatments used in preventive mode by spraying and watering showed a complete inhibition of the sporulation (100%) on *P. infestans* isolates and potato's varieties.

Discussion

Plants are able to produce various compounds. Besides the classic primary metabolites, they synthetize and accumulate secondary metabolites which the physiological function is not always obvious but represents a wide range of exploitable molecules in agriculture within the framework of phyto-protection.

R. officinalis antibacterial and antifungal activities can be summarized by the oil composition of these extracts [16]. The study revealed the phenolic compounds such as the terpenes, which include borneol, camphore, 1,8 cineole, pinene camphone, verbenonone and bornyl acetate [17].

This study as well as previous reports confirms the efficiency of certain extracts of plants in the control of potato mildew [18].

Several authors have also asserted that *R. officinalis* aqueous extract has a powerful antioxidant activity, associated with the presence of several di-terpenes phenolic as the carnosique acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol [19].

Besides, some reported that a number of compounds contained in the extracts of *R. officinalis* revealed antibacterial and antifungal properties [20,21].

So, the antifungal activity of *Rosmarinus officinalis* L. extracts was estimated against the fungal infections of wheat. Their inhibitory effect on the mycelial growth asserted that their use in low concentrations could have a significant potential for the biological control of phytopathogen fungi such as *Alternaria alternata, Botrytis cinerea and Fusarium oxysporum* [22].

On the other hand, Goussous et al. [23] reported important *in vitro* inhibitory effects of the crude extracts of various concentrations of *R. officinalis* against *Alternaria solani*. In addition, the microscopic observations of the fungal isolates treated by the extracts of *R. officinalis* revealed structural modifications leading to the lysis of the mycelium and, the digestion of sporangia contents, there by confirming the fungicidal effect of the plant extract. It is the toxic effect of its components on the structure and the physiology of the cellular membrane that is responsible for the antifungal effect [24,25]. In this sense, Omidbeygi et al. [26] suggested that the components of essential oil and extracts of plants cross cell wall and interact with enzymes and proteins, it is the production of a flux of protons towards the exterior cell which incites cellular changes and after all, the death of the microorganism. Cristani et al. [27] suggested that the antimicrobial activity is connected to the capacity of terpenes to affect not only the permeability but also the other functions of cell wall.

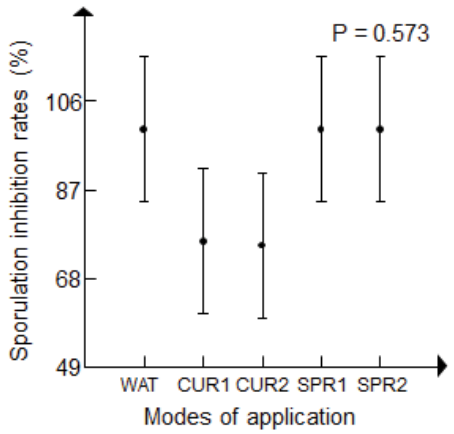
Our results revealed that all the extracts of *R. officinalis* even in the lowest concentration (5%), were excellent inhibitors of *P. infestans* sporulation and germination. Their inhibition rates were as high as 100%. This could have been caused by the deformation of sporangia and the lysis of the contents by the bioactive molecules contained in this aqueous extract.

It has further proved that the extracts of plants are rich in phenols also showed, an inhibitory activity particularly raised against the sporulation of fungi [28].

Duru et al. [29] confirmed that the antifungal effects of several extracts of plants seem to have a correlation with the incomplete

Para-meters	Application Modes of treatments	Sum of squares	d.d.l	Mean square	Test F	P
Modes of application	All the modes	2887.749	4	721.937	0.750	0.573
Varieties	Curative	1394.314	1	1394.314	0.678	0.457
P. infestans isoltates	Curative	5899.765	1	5899.765	2.867	0.166
Concentrations	Curative	127.417	1	127.417	0.062	0.816

Table 4: Variance analysis of sporulation inhibition rates according to treatments application modes, concentrations, varieties and *P. infestans* isolates.



WAT: Watering by the crude extract SPR1: spraying with crude extract SPR 2: spraying with diluted extract at 20% CUR1: curative treatment with crude extract CUR 2: curative treatment with diluted extract at 20%
Figure 6: Variance analysis of sporulation inhibition rates in GLM according to treatments application modes.

development of conidiophores and morphological modifications of *Aspergillus fumigatus*.

In the same context, Blaeser and Steiner [30] showed that the extracts of various plants prevent the germination and affect the release and the mobility of *P. infestans* zoospores, in agreement with this study.

In vitro and in vivo survivability of *P. infestans* isolates showed a fungistatic effect of *R. officinalis* with low concentrations and a fungicidal effect at increasing concentrations. The latter was able to prevent completely the appearance of the late blight symptoms on leaf disk of potato. The CIL value allowed us to know from which concentration the extract of the rosemary becomes fungicidal. Based on the scale of Koba et al and Webster et al. [31,32], we can deduce that *R. officinalis* extract has an interesting inhibitory power for A1 isolate because the A2 CIL exceeds 70%.

These results could be connected to the phytochemical composition of the plant. They suggest that it contains molecules with fungicidal activity. This hypothesis coincides with several studies reported by the bibliography. Indeed, Banso et al. [33] revealed fungistatic antifungal substances in the extracts of plants in low concentrations but, which become fungicidal in higher concentrations.

Conclusion

Results from this study as certain that aqueous extract of *R. officinalis* powder was an excellent inhibitor of *P. infestans* mycelial growth, sporulation and germination in the lowest dilution (5%). This could have resulted from the action of bioactive molecules contained in the rosemary aqueous extract, that causes the lysis of the mycelia and sporangia of the fungus. The fungicidal effects increased with increasing concentrations of this aqueous extract.

On the other hand, the aqueous extract of *R. officinalis* led to a reduction of the disease on leaf disk of potato. Important disease reduction rates (over 70%) were registered in both varieties and for both isolates of *P. infestans* exceeding 75%, while Spunta variety showed a more important reduction for treatment preventive application modes by watering (85%) and for the curative mode (90%). Also, the A2 isolate was greatly inhibited by treatments application rates of spraying (86%), watering and curative (83%). The sporulation inhibition was very pronounced *in vivo* (75% and 100%) and as the rates of preventive treatments made by spraying and watering. This present work thus confirms the bio-fungicidal potentialities of aqueous extract of *R. officinalis* on *P. infestans* isolates with the aim of its use in the bio control of late blight potato.

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