RESISTANCE OF PEPPER (*Capsicum annuum* L) TO *Phytophthora capsici* Leon. POWER AND LIMIT

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Résumé

Pour réaliser une sélection variétale, les variétés de poivron (*Capsicumannuum* L) en Algérie, ont été inoculées par des isolats locaux du champignon *Phytophthora capsici* Leon, et utilisé un test quantitatif. Les mesures des nécroses et des plantes mortes sont significativement différentes entre variétés et entre isolats dans les deux niveaux 0.05 et 0.01 : la variété "Italico II" a montré moins de sensitivité sur touts les organes avec des moyens 56.61 mm pour la tige, 30.27 mm pour la feuille, et 14.44 % des plantes mortes ; alors que la variété "Esterel" a enregistré plus de sensitivité sur touts les organes avec des moyens 122.91 mm pour la tige, 49.10 mm pour la feuille, et 70% des plantes mortes, et les autres variétés sont graduellement ordonnées et médianes aux deux extrêmes variétés. L'isolat 3 de la région Jijel a apparu une grande agressivité sur touts les organes de plante. On propose l'utilisation des variétés moins sensibledans l'agriculture, puisqu'elles ont de qualités qui satisfait l'agriculteur et le consommateur, ou bien chercher des variétés vraiment résistantes dans toutes les conditions, ouessayer de diminuer l'inoculum du champignon par des cultures de rotation.

Mots clés : *Sélection variétale, Résistance, Capsicumannuum L, Phytophthora capsiciLeon.*

Abstract

To realize a varietal selection, the cultivars of pepper (*Capsicum annuum* L) in Algeria, were inoculated with the local isolates of the fungus *Phytophhoracapsici* Leon, and used a quantitate test. The measures of the necrosis and died plants were significantly different between cultivars and isolates at the two levels 0.05 and 0.01: the cultivars "Italico II" showed lower sensitivity on all plant organs with means 56.61 mm for the stem,30.27mm for the leaf, and 14.44% died plants; while the cultivar "Esterel" scored higher sensitivity on all organs also with means 122.91 mm for the stem, 49.10mm for the leaf, and 70% died plants, and the other cultivars had agraduate order and were median these two extremes cultivars in sensitivity. Isolate 3 of Jijel area expressed great aggressiveness in all organs of plant. We suggest using the lower cultivars sensitivity especially since it has qualities to the grower and the consumer, or looking forreally resistant variety in different conditions, or try to reducetheinoculumof the fungus by crops rotation.

Keywords : *Cultivars selection, Resistance, Capsicum annuum L, Phytophthoracapsici Leon.*

ملخص

لتحقيق انتخاب صنفي، اخذت مجموعة من أصناف الفلفل الحلو (*Capsicumannuum*L.) الموجودة في الجزائر، ولقحت بمجموعة من عزل الفطر *Phytophthora capsici*Leon المحلية. قياسات النيكروز (الموت الموضعي للأنسجة) والنباتات الميتة اختلفت بفروق معنوية بين الأصناف وبين العزل في كلا المستويين 0.05 و0.01. اعطى الصنف "Italico II" اقل حساسية في كل اعضاء النبات وذلك بمعدل 56.61 ملم في الساق، 30.27 ملم في الورقة، و 14.44 % نباتات ميتة من جراء حدة اصابة الجذور بالتعفن. قدم الصنف "Esterel" أكبر حساسية في كل الأعضاء وذلك بقياس متوسط 122.91 ملم في الساق، 49.10 ملم في الورقة، و 70 % نباتات ميتة. باقي الأصناف كانت في ترتيب تدريجي ووسطية بين هذين الصنفيين الحدين. سجلت عزلة 3 لمنطقة جيجل أكبر عدوانية. يقترح مواصلة زراعة الأصناف الأقل حساسية لأن لها صفات مورفولوجية مستحسنة من طرف المزارع والمستهلك، أو البحث عن أصناف حقيقية مقاومة في كل الظروف البيئية، أو محاولة تخفيض لقاح الفطر بعمل دورات زراعية.

الكلمات المفتاحية: *انتخاب الأصناف، المقاومة، Capsicumannuum L، Phytophthora capsiciLeon*

Pepper (*Capsicum annuum*L), is a food crop and source of international trade [1]. The importance of its surface and its extension in the world and in Algeria has exposed cultures to aggressive and very many cosmopolitan parasites, among which are found in head *Phytophthoracapsici* Leon fungus [2;3], it is the agent of late blight for many families[4], especially for the *Solanaceae* and the *Cucurbitaceae* [5]. The danger of this disease and its agent causal is manifested by the appearance of several symptoms with various forms of alteration in all organs, the rapid dispersion of pathogen between the organs and all environments life, and its types of reproduction producing different sources of contamination [6].

There are several methods or strategies that have been implemented to combat the fungus *P.capsici* Leon, among which a more widespread throughout the world and in Algeria, is chemical control has focused on the use of pesticides under greenhouse or fieldconditions[7], but unfortunately these products have drawbacks on health and environment [8; 9], and it seemed that this fungus has a rapid tolerance to these products which became inadequate in controlling it [10].

Today, there are enormous efforts are devoted to research natural defenses for the plant or stimulators (SDN) capable of initiating the molecular, biochemical, an cellular events that lead to expression of plant resistance, and how to use them in eco diversity[11].The majority of breeders and pathologists researchers who were interested in protecting crops are oriented towards genetic resistance [12], developing cultivars carrying effective resistance became priority while little is currently known about the genetic basis of durable resistance [13].

The results accumulated for several years of research either in natural conditions or artificial contamination confirmed that the resistance of pepper against the fungus *P.capsici* Leon is polygenic, speaking part, and focused on parents" PM217", resulting from successive self-fertilization of pepper " PI201-234 " according to the researchers Kimble and Grogan (1960)[14], and has remained the parent first and best source although there are several sources that are available with a certain degree of resistance [15], and the researchers Mallard el al(2013) [13] propose that QTL is a key element responsible for the broad-spectrum resistance to *P.capsici*, it's a valuable locus for improving the effective resistance.

Scientific selection for commercial varieties is recent[16], its objective is to exploit the diversity desired [17] or to achieve disease-resistant varieties [18]. The results of this selection gave a large number of hybrids (F1), sterile and non-constant, which is why their use is difficult [19].

Algeria remains dependent to the external market despite their effort to create stations to produce and store seeds. In this respect, it imports seeds of some European and Asian countries, which it carries pepper morphological and physiological variability and even pathological, with different levels of resistance, especially to the fungi *Phytophthoracapsici* Leon, and as its interaction with the pepper (*Capsicum annuum* L) is dynamic and quantitative, and that the resistance is recognized by the comparison between varieties under conditions, therefore it is necessary to make an analytical study of these cultivars introduced to determine their limits of resistance vis-à-vis local isolates of *Phytophthoracapsici* Leon, and the power for this pathogen.

MATERIAL AND METHODS

Plant material

The plant material subjected to the study is represented by fourteen cultivars of sweet pepper (*Capsicum annuum* L) (Sweet Algiers, Sonar, Esterel, Sweet Marconi,Predi, Magister, Belconi, Italico II, Lipari, Arabal, Sweet Spanish, Bruyo, Andalus and Pacifio). Usually they are grown in greenhouses in Algeria, mainly in the region of Jijel. Most cultivars are commercial hybrids except Sweet Spanish, Sweet Marconi, and Sweet Algiers are stable (ITCMI, 2001)[20].

According to classification of sweet pepper suggested by Nuez et al (1996) [1], which has adopted by European countries and countries located in West of Mediterranean such as Algeria, it has based on morphological criterions, our cultivars tested belong to rectangular phenotypes (Pacifio, Predi, Sonar, Esterel, Andalus, Bruyo and Magister) or triangular phenotypes (Sweet Algiers, Sweet Marconi, Belconi, Lipari, Arabal, Italico II and Sweet spanish). They were prepared in the following steps:

Seedlings were prepared in containers of small pots (4^2X6.5) cm^3, witch were filled with a culture medium consisting of a sterile black peat (2/3 volume) and a proper silica sand (1/3 volume), then were seeded and incubated at temperature 20 ± 2°C. After a month and half of growing [21], one group of seedlings were transplanted in a solid medium: it is a larger pots (12^2X21) cm^3 containers a fertile organic soil was taken from a soil profile (Hz : O and A) of the cork oak forest of Taza-Jijel, sterilized by steam, and its measurement of pH is about 6.7. Another group of seedlings were transplanted in a liquid medium were poured in Pasteur flasks: it is a mixture of 150 ml of NPK (15/15/14) with concentration 1.5g / L added to 50 ml of Richard medium, the measurement of pH is about 7.8.

Fungal material

The scanner fields were conducted in greenhouses located in ecologically different areas in Algeria: the humid region - Jijel, the semi-arid region- Constantina, and arid region - Biskra. These areas are known for their vegetable crops, especially pepper (*Capsicum annuum* L) and their parasites attacks such as *Phytophthoracapsici* Leon. In order to isolate and pure this fungus we have collected and gathered fruits and roots rotten and necrotic leaves and

stems according to the symptoms described by researchers Leonian (1922) [22], Satour and Butler (1967) [23].

The isolation from aerial organs was by cutting fragments from infected areas in stems, fruits and leaves. These fragments were washed with normal water, disinfected with ethanol (95%), rinsed with sterile distilled water, and dried by a filter paper, after its were inoculated onto the surface of the medium V-8 poured into Petri dishes. This preparation was incubated at 28°C temperature for a week, after we did a whole subculture of colonies for homogeneous shape, appearance and color [24]. To accomplish the isolation and purification of *Phytophthoracapsici* Leon from roots we had applied the technique Satour and Butler (1967)[23].

After the determination of those different isolates witch was carried out according the morphological and physiological criterions of*Phytophthoracapsici*fungus cited by a group of researchers in theirs descriptions, keys, classifications, and reviews[22, 25, 26, 27, 28] we had complemented by their power pathogens and obtained six isolates different in theirs origin:

Isolates (3, 1) were from Jijel region; 3 isolated from rotten roots, 1 isolated from spotted leaves.

Isolates (4, 2) were from Constantina region; 4 isolated from rotten roots, 2 isolated from rotten fruits.

Isolates (5, 6) were from Biskra region; 5 isolated from rotten roots, 6 isolated from stems necrosis.

The pure isolates were transplanted in organic medium (V-8) every month and conserved at 5°C.

Artificial contamination

This step is very important to estimate the resistance of pepper. Experiments of artificial contamination on organs of different cultivars of *Capsicum annuum* L. by isolates of *Phytophthoracapsici* Leon were conducted in a climatic chamber at temperature 22 ± 2 °C, 12 hours light daily, 100% relative humidity or approximate, cultures of the fungus that does not exceed the age of 10 days, and the majority of plants are in the vegetative phase [29, 30].

Measuring the power of the fungus and the resistance limits on the following organs:

Stems

This study was performed on fourteen cultivars of pepper (*Capsicum annuum* L) cited in precedent, which were transplanted in the solid medium. At age month and half of plants, we had applied Pochard*and al.* (1976) [31] method, which has always been appreciated and used by researchers breeders: the stems were decapitated under the last leaf, the inoculum was added in the disc form (Ø = 4 mm) of the fungus pure culture *P.capsici* Leon (isolates 1, 2, 3, 4, 5 and 6), covering the entire cross section of each stem,and an aluminum foil was used to maintain high humidity at the top of the stem to form a humid chamber. The interest variablewas the length of the external necrosis induced by the fungal isolates as to their progression in the stem. The measurement of the extension of necrosis was made for15 days.

Leaves

Resistance measurement was made on adult leaves of pepper (*Capsicum annuum* L) cultivars cited in precedent, faced to isolates 3, 4 and 5 of the fungus *Phytopthoracapsici* Leon, which expressed great aggression on the stem, and coming from the three ecological region cited in precedent in Algeria. The leaves were plated in Petri dishes on a thin membrane of sterile distilled water, inoculated in the middle of their main veins with a wing needle platinum by a mycelia disc (Ø = 4mm), after the Petri dishes were closed to keep their moisture. The estimation of the resistance of the leaves was performed by measuring the extension of necrotic spots from the point of inoculation to limit after five days [32].

Roots

The level of resistance in the roots was studied on cultivars of sweet pepper cited in precedent, against isolates 3, 4 and 5 of the fungus *P.capsici* Leon. Before transplanting plants in the liquid poured into Pasteur bottles, four discs (Ø = 4 mm) of the fungus were thrown into this medium late release motile zoospores that move toward the roots. The evaluation of the resistance in the roots was measured in all plants, was estimated by percentage mortality of plants after 15 days, we judge that the plants died when they affect a wilting and drying after a root or root collar seriously by researchers Satour and Butler (1967), Yildiz and Delen (1979) [23], [7].

Statistical analysis

Math's statistic was necessary to analyze our results. The experiment on the stem was carried on factorial distribution, but on the leaf and root was random distribution. ANOVA tables were created. When F (variance of the treatments / variance of values) was superior to F in the Fisher's table at 5% or 1% level, Fisher's LSD test was necessary to know the significant differences between the means of treatments when its compared with the low significant difference (LSD) at freedom degrees (Df) of the values and 5% or 1% level. This test was used when the number of treatments fewer than 5, but above 5 treatments Duncan's LSR test was important to know the significant differences between the mean's treatments when we compared with the low significant range (LSR).

RESULTS

Evaluation of the resistance in stems

The interaction of the fungus *P.capsici* Leon with sweet pepper (*C.annuum* L) was significant (Df: 167/336, F = 809.403) in the two levels 0.05 and 0.01: the answer stems cultivars against isolates of the fungus resulted in the appearance of brown necrosis extended from the top to the base of each stem with different length and speed which was constant in each stem throughout the infection, and varied from 8.19 to 3.77 mm/day (Fig 1).

Figure 1: Expanded necrosis from the top to the base of sweet pepper stems by *Phytophthoracapsici* Leon.

The main effect of genotype in the development of resistance, whose answers necrotic cultivars chosen in this study were significant (Df: 13/336, F =8518.618) in the two levels 0.05 and 0.01: the necrotic lesion was taller in the variety" Esterel" than all varieties with an average length 122.91 mm; but it was smaller in the variety "Italico II" with an average length 56.61 mm (Fig 2).

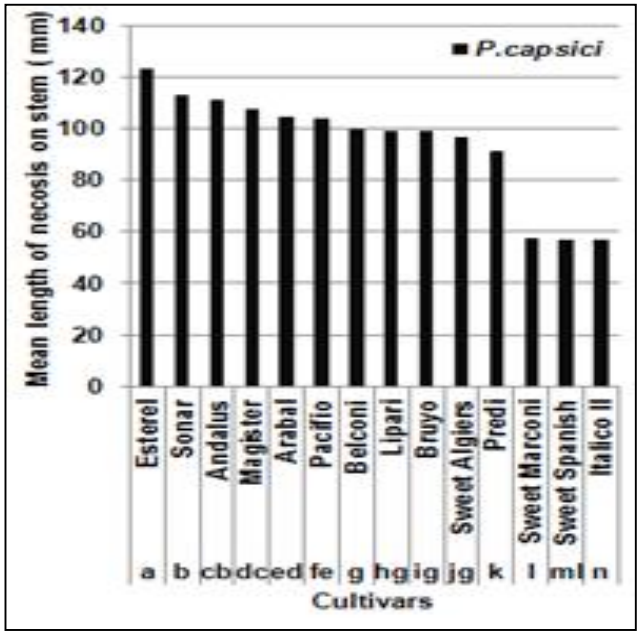

Figure 2: Effect of different cultivars on resistance of sweet pepper stems against *Phytophthoracapsici* Leon fungi.

The different of histograms length and lower-case letters express the mean necrosis length for each cultivar under effect of all isolates according to Duncan's test. For example a and n sign the significant difference between the meansof cultivars Esterel and ItalicoII, but b and cb sign that there is no significant difference between cultivars Sonar and Andalus compared with LSR.

The effect of fungal isolates tested is very important in the variability of resistance, their influence was significant (Df: 5/336, F =1366.299) in both levels0.05 and 0.01: Isolate 3 of Jijel region marked a taller necrosis with length 108.09 mm; while isolate 6 of Biskra region marked a lower necrosis with length 78.03 mm(Fig 3).

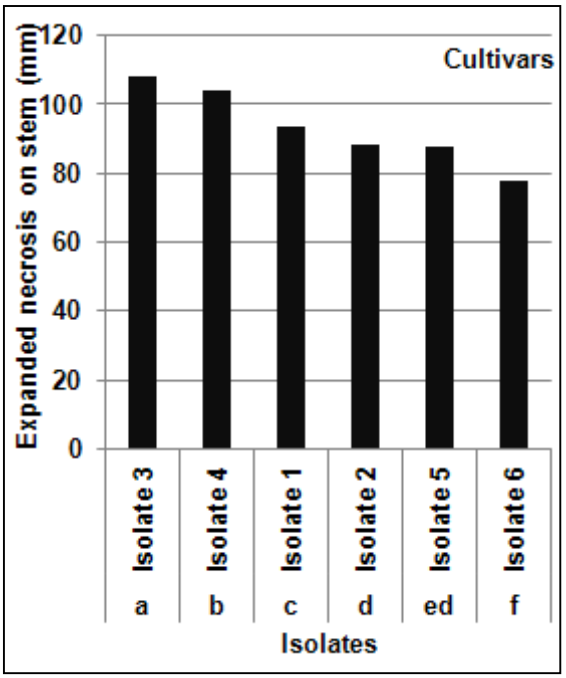

Figure 3: Effect of *Phytophthora capsici* Leon isolates on resistance of sweet pepper stems.

The different of histograms length and lower-case letters sign of mean length of necrosis for each isolate under the effect of all cultivars according to Duncan's test. For example a and f sign the significant difference between isolate 3 and 6, but d and ed express no significant difference between isolate 2 and 5 compared with LSR.

Evaluation of the resistance in the leaves

Artificial inoculation on pepper leaves, gave a mollnecrotic spots with constant speed throughout the infection, varied from 9,82 to 6,05 mm/day,took a brown color in the end of infection(Fig 4), and they noticed significant differences (ddf: 13/210, F =365.047) at the two levels 0.05 and 0.01: after 5 days, the larger spot necrosis was formedin the cultivar "Esterel" with an average diameter 49.10mm; while the smaller spot necrosiswas formed in the cultivar "Italico II" with an average diameter 30.27mm, and the remain cultivars were intermediate these

two extreme cultivars (Fig 5).The expressed infection by *Phytophthoracapsici* Leon isolates was significant (Df: 2/210, F=1447.183) at the two levels 0.05 and 0.01, depending the isolate : isolate 3 gave a widest spot necrosis with diameter 42.25mm (Fig 6).

Figure 4 : Development of spot necrosis on sweet pepper leaves by *Phytophthora capsici* Leon.

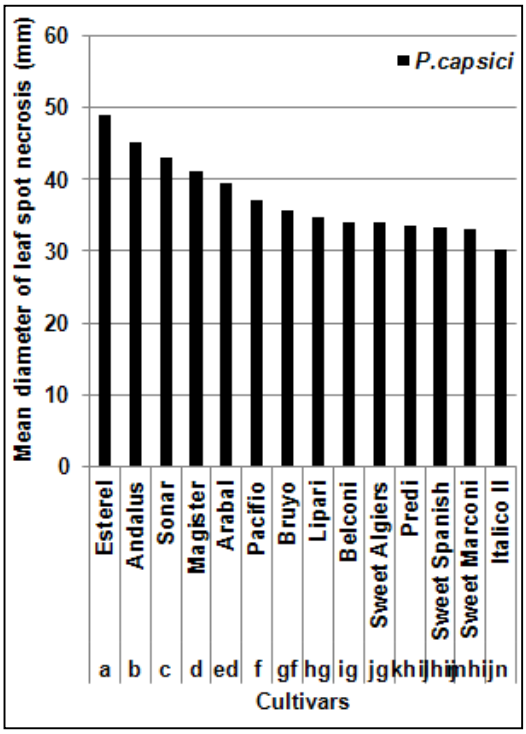

Figure 5: Effect of different cultivars on resistance of sweet pepper leaves against *Phytophthora capsici* Leon fungi.

The different of histograms length and lower-case letters express the mean diameter of spot necrosis for each cultivar under effect of all isolates according to Duncan's test. For example a and jn sign the significant difference between the cultivars Esterel and Italico II, but d and ed sign that there is no significant difference between cultivars Magister and Arabel compared with LSR.

Evaluation of resistance in roots

Exposure roots of fourteen cultivars of pepper in inoculation with three isolates (3, 4 and 5) of the fungus *P.capsici*Leon responded with the admissibility of roots or roots – collars to infection: tissues roots, at different points were exposed to destruction and rot, then formed a brown-gray necrosis, extended to the entire root or reached the crown of the plant, after the plants wilt and dry, so it is the death of plants (Fig 7).

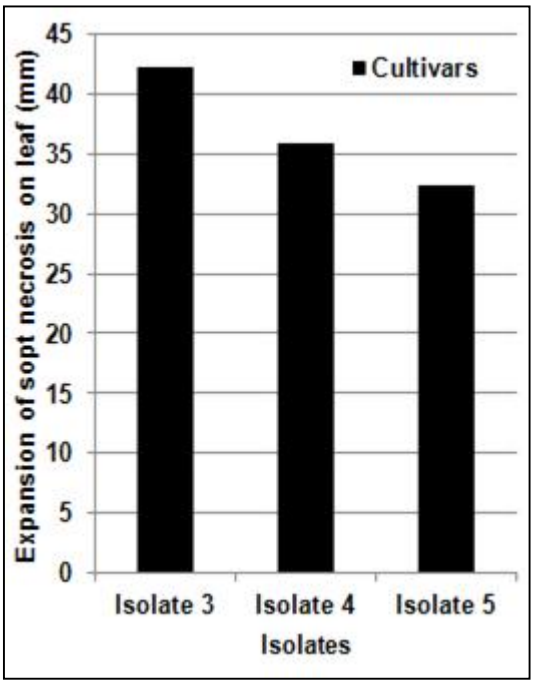

Figure 6: Effect of *Phytophthora capsici* Leon isolates on resistance of sweet pepper leaves.

The difference of histograms length sign the mean diameter of spot necrosis for each isolate under the effect of all cultivars, and it was significantly different when we compared with LSD.

Figure 7: Root and crown rot, foliar system wilting and drying of sweet pepper by *Phytophthora capsici* Leon.

By calculating the percentage mortality of infected plants through their roots or roots-collars, it differed significantly depending the cultivar (Df: 13/84, F =15,884) at the two levels 0.05 and 0.01. The comparison between the means of the percentage of infection of different cultivars gave the following results: the cultivar "Italico II" less eligibility to infection with an average percentage of died plants 14.44 %, the cultivar "Esterel" great admissibility to infection with a mean percentage of died plants 70%, but the remain of varieties were median in infection (Fig 8).

The different of histograms length and lower-case letters express the mean mortality of plants for each cultivar under effect of all isolates according to Duncan's test. For example a andcbsign the significant difference between the cultivars Esterel and Sonar, but a and basign that there is no significant difference between cultivars Esterel and Andalus compared with LSR.

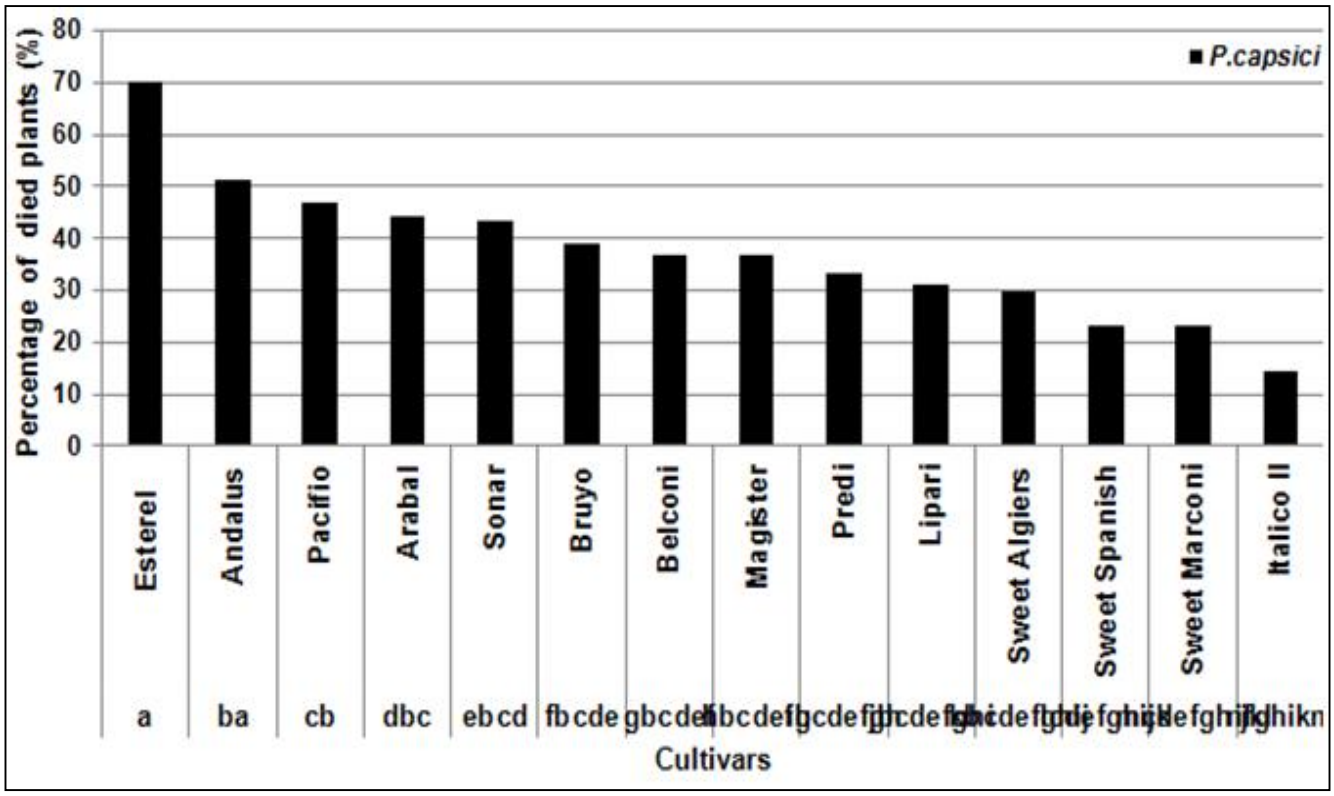

Figure 8 : Effect of different cultivars on resistance of sweet pepper roots against *Phytophthoracapsici* Leon fungi

The progression of disease by various isolates of *P.capsici* Leon differentiated significantly (Df: 2/84, F=70,955) at the two levels 0.05 and 0.01: isolate 3 was marked a higher percentage of died plants (47.61 %); while isolate 5 was marked a lower percentage (22.14 %)(Fig 9).

The different of histograms length sign the different between percentage means of mortality plants induced by different isolates and it was significantly when we compared with LSD.

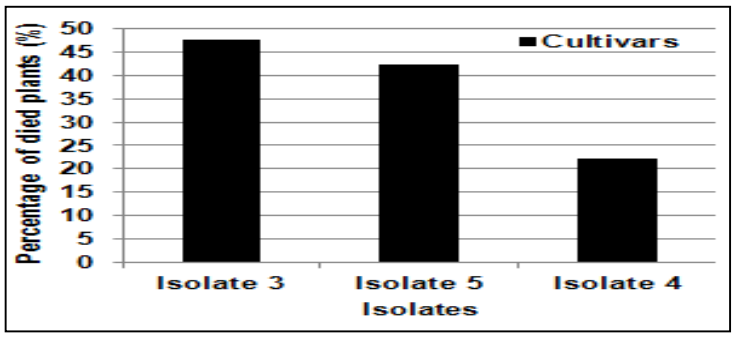

Figure 9 : Effect of *Phytophthoracapsici* Leon isolates on resistance of sweet pepper roots.

DISCUSSION

Phytophthora capsici Leon, is a soilborn fungus, can invade the crown and roots of pepper (*Capsicumannuum* L) [30], causing a slimy soft rot, is characterized by tissue destruction, which then undergoes a dryly and the color transformed to dark brown [33]. The infection of the root and crown ends with a sudden wilting of leaves system without yellowing, after there will be sharply [7] and death of the plant in a period not exceeding 15 days. Necrosis may occur in the stem of the pepper from the summit to the base intermediate the spores of *P.capsici* Leon carried by the wind at the top of the stem [23].

The leaves of peppercan also infect in all phases of their development by the pathogen *P. capsici* Leon, it has the ability to diffuse into the tissue of the leaf blade and leaf veins in forming brown necrotic spots with various shapes [32]. At first the spots are wet after their change in color yellow and brown in the end[34].*P. capsici*can also invade other susceptible hosts and causes foliar blighting, wilting, root, stem and fruit rot [5].

Artificial inoculation, on various organs (stems, leaves, and roots), of pepper(*Capsicum annuum* L) cultivars, with a range of isolates of *Phytophthora capsici* Leon, in condition of chamber culture, appeared symptoms similar to those described previously by researchers, have dynamics and quantities symptoms by which we could estimate the resistance in the cultivars tested and compared according to their intensity and power of each isolate.

In the root, we tried to measure its resistance in the group, by counting the percentage of plant mortality: the root is an underground organ, which can not really see the changes and developments of natural decay, except that the deduct, the root is also brown color, which we could not practically distinguish and identify small areas of infection, and as the root consists a five different areas in tissue and age, as well as the water solution containing the spores where we introduced the root is all around, can infect it at any point and at any time, and all its components make the value of the resistance individually into the roots via the intensity of symptoms rotten necrotic difficult.

The inoculation on various organs of different pepper cultivars showed different degrees of sensitivity to the fungal isolates, resulting in hypersensitive being expressed along the organs with constant speed. The heterogeneity of resistance responses due to the heterogeneity of genetic material plants[31].

The sensitivity of these cultivars explains the significant losses in pepper's green houses of Algeria, especially in rainy weatherand bad controlling irrigation ,but in the non-rainy weather and with good control culture by using chemical products, cultivars pepper planted in Algeria showed an acceptable level of resistance which satisfied the major of Algerian growers pepper, it confirms that there is no absolute resistance, and it changes with cultural conditions. The expression of resistance in the cultivars tested was discontinuous between the organs in some cultivars and continuously in others, which confirms the variability of resistance in different part of the same plant.

Resistance in pepper is a phenomenon induced by the pathogen [35], controlled by genes [36]. Resistance to root and crown rot in pepper are governed by different genetics mechanisms than resistance of fruit and foliar blighting [37], and related by enzyme activities [38]: peroxidase [39] which stimulates the formation of carbohydrates to thicken the cell wall at the point of confrontation with the fungus, forming a barrier that stops penetration called "opposition wall " [40], and also catalyzes and phenolic compounds [41].

In pepper family *Solanaceae*, biotic and abiotic elicitors induce de novo synthesis of sesquiterpenoids, stress metabolites known as phytoalexins, because plant hormones play criticalroles in the induction of defense responsive genes [42]. Capsidiol, aphytoalexin which accumulates in the area of necrosis appears to be involved in the resistance [43], it stops the action of the fungus in early stages infection [44].

The fungus *P.capsici* Leon also includes chemical means which enable it to invade any variety with less or ease according to the nature of the tissue and invasive strain [45].

Enhancing the host resistance by using naturally occurring elicitors derived from pathogenic organisms is an emerging as an ecofriendly approach in plant management [38]. Future breeding efforts should focus on developing cultivars that display resistance to set of isolates that encompass the genetics and virulence [46] diversity within *P. capsici* host resistance screens are affected by host type and plant part screened, pathogen culture, storage condition and environmental conditions during the screening [46, 47].

CONCLUSION

Any illness is the result of a struggle between two beings: the parasite and the host plant. The parasite is represented by the fungus *Phytophthora capsici* Leon, is characterized by its aggressiveness, it's all attack powers available to it to enter the body of its host plant, which is represented by the sweet pepper (*Capsicum annuum* L), the latter is constituted by cells that respond to infection.

The reactions are sometimes considered to prevent or limit the growth of the fungus and are resistance factors. The artificial inoculation of the various organs of some commercial cultivars of pepper by a spectrum of local isolates its purpose to order and select the best commercial sources of resistance, which has been sorted cultivar "Italico II" as the lower sensitive source and the cultivar "Esterel" as a higher sensitive source, and other cultivars are intermediate these two extremes cultivars, without forgetting that the isolate 3 of Jijel-Algeria is the most

aggressive. This analysis was made through the evaluation of the severity of symptoms in different organs which are quantitative and kinetic in pepper. We can give importance to the least susceptible cultivars in agriculture as they have a certain level of resistance in certain circumstances, and they have desirable characters for consumer.

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