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Effects of four plants and solarization on bacterial wilt of tomato caused by *Ralstonia solanacearum* E. F. Smith in Burkina Faso

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Bacterial wilt caused by *Ralstonia solanacearum* is a major constraint in tomato production. Experiments were carried out using four sanitised plants and solarization in a semi- controlled environment and in the field to reduce the infectious potential of the soil in *R. solanacearum*. The experimental design used is a randomized Fisher block with eight (8) treatments composed of *Ocimum basilicom*, *Ocimum gratissimum*, *Allium cepa*, *Crotalaria retusa*, solarization, untreated control, bactericide (IDEFIX) and the biocontrol indicator (Rossol). Seventy days after the implementation in the field, the initial infectious potential of 1.07×10^{8} CFU g^{-1} of dry soil increased to 4.11×10^{7} CFU g^{-1} of dry soil, an average reduction of 55.63%. *O. gratissimum* is the best sanitizing plant with 68.18% reduction in the infectious potential of the soil. In a semi-controlled environment *C. retusa* recorded the greatest reduction (73.96%) of the infectious potential of the soil among the sanitizing plants. The greatest reductions in disease incidence in the field were observed with solarization (60%) followed by *C. retusa* (58%).

Key words: *Ocimum basilicom*, *Ocimum gratissimum*, Infectious potential, Sanitizing, tomato, semi-controlled environment.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is one of the major biotic contraints of tomato (Mansfield et al., 2012). This bacteriosis can cause losses of more than 90% in tomato cultivation (Ouédraogo and d'Arondel, 1994). The control of this disease represents a major challenge for market gardeners. Cultural, biological, genetic and chemical control have been investigated to control this disease. The use of synthetic chemical pesticides degrades the environment and human health (Wu et al., 2012). Faced with this threat, it is imperative to

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consider appropriate and environmentally friendly solutions such as the association of tomatoes with aromatic plants (Bianchi et al., 2006; Son et al., 2018). Indeed, sanitizing plants and solarization have given significant results on the disease in Martinique (Fernandes et al., 2012; Launay, 2012). It is within this dynamic that the sanitizing effects of four local plants and solarization were evaluated on the manifestation of the disease to increasing tomato production.

MATERIALS AND METHODS

Experimental sites

The study was conducted in a semi-controlled environment at the bacteriology laboratory of INERA Farako-Bâ (11° 09' 21.6" Latitude North and 004° 17' 09.7" Longitude West) and in the open field on the market garden site of a producer in Toussiana located 53 km from Bobo-Dioulasso (10° 50' 32.4" Latitude North and 004° 39' 36.6" Longitude West).

Plant material

The plant material used was composed of four local plants (*Ocimum basilicum* L., *Ocimum gratissimum* L., *Crotalaria retusa* L. and *Allium cepa* L.) and rossol variety of tomato. It is a short cycle variety (80-90 days) and with a fixed habit (FAO, 2008). It adapts to the agro-climatic conditions of the region and can be produced in any season. The choice of this variety is due to its sensitivity to bacterial wilt, and its tolerance to *Verticillium, Fusarium oxysporum* and nematodes (V.F.N).

Pathogen used

The pathogen used in a semi-controlled environment is the local strain NMDG 111 (Phylotype I/ Sequevar 31) of *R. solanacearum* with an aggressiveness of nearly 100 (Traoré et al., 2022). In the open field; the infestation was natural.

Fertilizers and phytosanitary products

The organic manure used was compost made from cow manure at a dose of 18 T ha^{-1}. NPK (15-15-15) at a dose of 300 kg ha^{-1} and urea (46%) at a dose of 200 kg ha^{-1} served as mineral fertilizers. Mancozeb (Dithane M 45) was used against fungi at 2 kg ha^{-1}, Cypermetrine (Cypercal 50 EC) against insects used at 1 L ha^{-1} and Profenofos (Arsenal 50 EC) against mites used at 1 kg ha^{-1} and Idéfix (65.5% copper hydroxide) used at 2 kg ha^{-1} as control.

Experimental setup

The trials were conducted in a semi-controlled environment (the growing medium was sterile and the pots were in trays) to assess the effect of the plants on the pathogen *in vivo*. The experimental design was a completely randomized block consisting of eight (08) treatments repeated five times. The plants were transplanted into pots containing culture substrates previously sterilized at 100°C for 30 min. The infestation consisted of infesting the injured roots of each plant with 15 mL of *R. solanacearum* inoculum at a concentration of 10^8 CFU mL^{-1}. The experimental field design was a randomized Fisher block, of eight treatments repeated in five blocks. Each block was composed of eight (08) modalities arranged randomly. The distances between the elementary plots (EP) were 0.5 m and 1 m between the blocks. Each EP was 5.76 m² (2.4 × 2.4 m) including 3.6 m² of usable area. The tomato plants were placed on ridges and each EP had 28 plants. The trial was conducted in three phases. The first phase of 70 days remained unchanged; the second phase consisted of cutting the plants to make mulch on the elementary plots and the third phase consisted of planting the tomato on all of the elementary plots. The transplanting was carried out in the evening after a good watering. The plants were rooted down to the collar and soil carefully packed around the roots. One week after transplanting, dead or faded plants were replaced with healthy plants. The maintenance focused mainly on weeding/hoeing, fertilization and phytosanitary treatment as needed.

Data collected

Observations were made on 10 mediums plants to avoid edge effects in the open field. Symptoms were noted weekly by counting wilted plants. This count began two weeks after transplantation. Disease progression was monitored over four weeks. The severity was noted on plants according to the scale of Coupat-Goutaland et al. (2011). The wilt index (WI) was expressed by the formula described by Jeger and Viljanen-Rollinson (2001):

$$WI = \frac{N}{Nt} x100$$

WI: Wilt Index; N: Number of wilted plants; Nt: Total number of plants observed.

$$\mathbf{AUDPC\ (t_k) = \sum_{k=1} (IF_i + IF_{i+1})\ (t_{i+1} - t_i) / 2}$$

AUDPC (tk) corresponds to area under the disease progress curve (disease progression kinetics) at × days after sowing/transplanting, IFi corresponds to IF on the previous day of observation, IFi+1 corresponds to IF on the day of observation, ti+1 corresponds to the rating date and ti corresponds to the date of the previous observation.

Evaluation of the infectious potential of market garden soils in the open field

Soil samples were taken from the six market garden sites with high production from *Solanaceae* crops area using the technique derived from Pochon and Tardieux (1962). Indeed, an average sample of 10 g of soil per elementary plot was collected at a depth of 10 to 20 cm. This collection was done in five (05) points following the diagonals of each plot. The samples were shaken at 250 rpm for 2 h in extraction buffer (0.85% NaCl) in the laboratory. 1 mL of each stirred sample was then used to prepare 4 decreasing concentrations (10^{-1},10^{-2},10^{-3} and 10^{-4}) in vials each containing 9 mL of sterile nutrient broth. Finally 10 µL of each dilution including the initial suspension were spread on semi-selective agar medium (SMSA) and incubated for 48 to 72 h at a temperature of 28 to 30°C.

The counting of the typical virulent colonies of *Ralstonia solanacearum* was followed using Pétri dishes according to the ISO 7218 (1985) standard. The determination of the number N of bacteria was determined by the following formula:

$$N = \frac{\sum C}{v * (n1 + n2 * 0.1) * d}$$

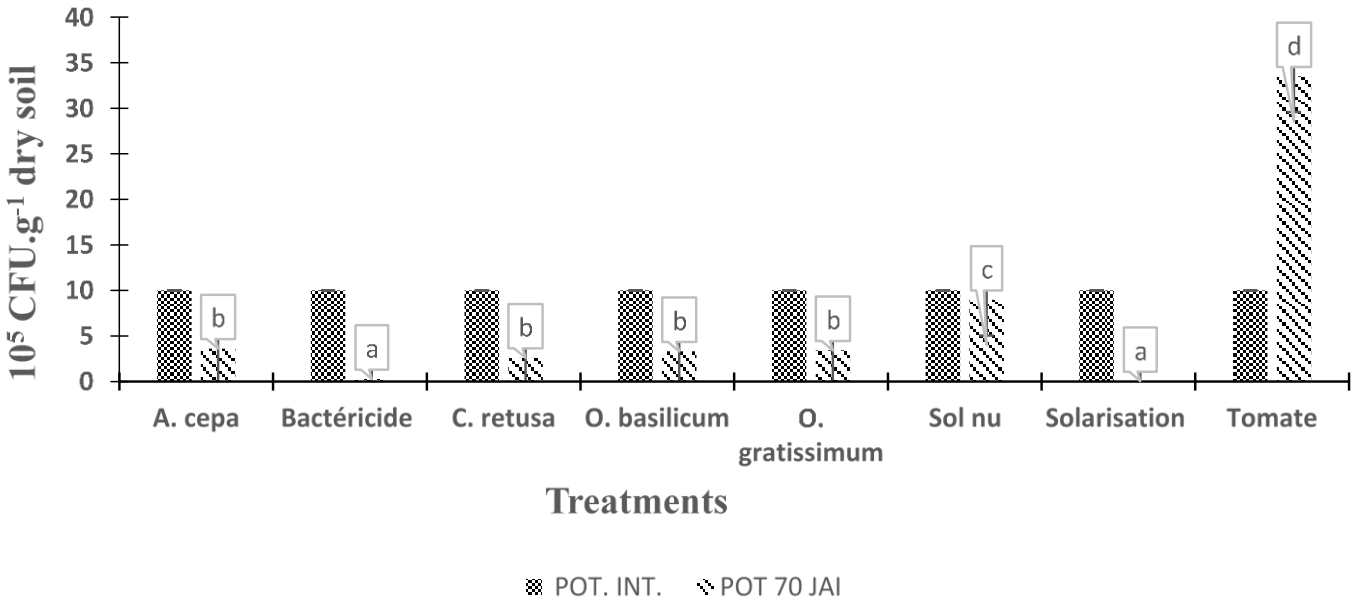

Figure 1. Effect of treatments on the inoculum potential of the soil in a semi-controlled environment. Column numbers assigned the same letter do not differ significantly at the 5% threshold (Newman-Keuls Test).
Source: Authors

∑C corresponds to the sum of the colonies counted, v being the volume of the solution used, d is the dilution of the 1st dish, n1 is the number of dishes of the 1st dilution used in the calculation, n2 is the number of dishes of the 2nd dilution and N is the number of bacteria in CFU mL^{-1}, 0.1 is the constant. Finally, the CFU g^{-1} of dry soil (Ns) is obtained by multiplying N by 10 on the humidity coefficient of the sample.

$$Ns = \frac{N*10}{(1-Hs)}$$

10 corresponding to the mass of moist soil used, Hs being the moisture coefficient and N being the CFU mL^{-1}.

Data processing

The data obtained was entered using an Excel version 2016 spreadsheet. This spreadsheet was also used to construct the graphs. The analysis of variance following the Newman-Keuls multiple comparison tests were carried out with the XLSTAT 2007.07.02 software at the 5% threshold.

RESULTS

Effects of different treatments on the infectious potential of the soil in a semi-controlled environment

The analysis of the results shows a very highly significant difference between the sanitizing treatments and the tomato (P = 0.0001). Indeed, a reduction of the innoculum of the order of 6.82 107 ± 2.94 107 CFU g^{-1} was notice (Figure 1). Moreover, the solarization and the bactericide respectively give the best reductions in the infectious potential of the soil (99.64 and 97.22%). Among the plants, the greatest reduction was obtained with *C. retusa* (73.96%).

Evaluation of the infectious potential of the soil in the field

Of the 40 soil samples taken, it appears that the soil of the experimental site is infected (Figure 2). Indeed, the inoculum rate is between 272×10^5 and 37×10^7 CFU g^{-1} of dry soil. The average infectious potential of said site is estimated at 1.07×10^8 CFU g^{-1} of dry soil $\pm8.05\times10^7$ CFU g^{-1}. The comparison of the means does not show any significant difference between the infectious potential of the elementary plots (P = 0.32).

Field disease incidence in pre-trials

During the pre-trial phase, the incidence of the disease was evaluated in the tomato plots. The analysis of Figure 3 shows a mortality of more than 50% from the 15th day after transplanting (DAT). This mortality evolved to reach 100% in the tomato plots at the 36th DAT.

Effects of different treatments on the inoculum potential of soil in the field

The analysis of soil samples in the field during 70 days after the implementation of the various treatments shows

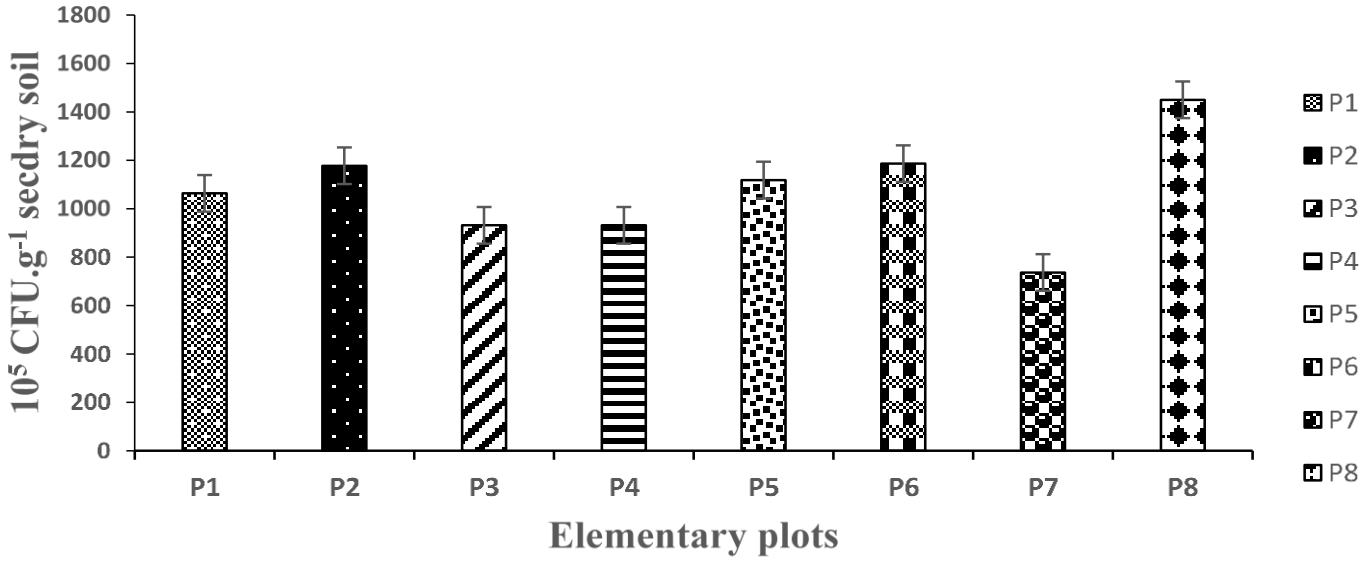

Figure 2. Initial inoculum potential of the experimental soil.
Source: Authors

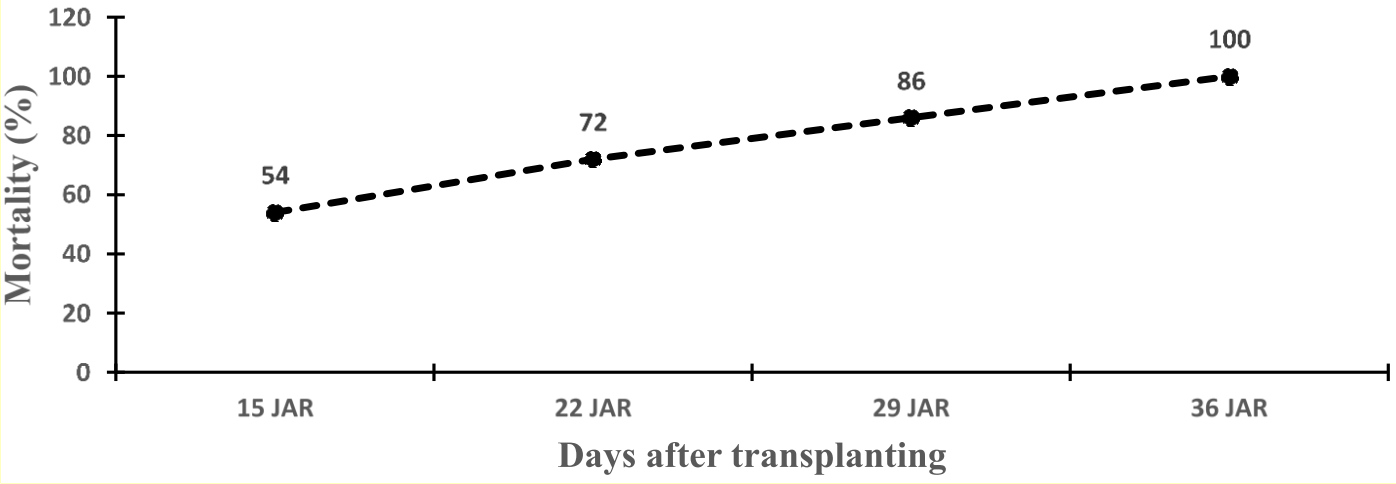

Figure 3. Progression of cumulative mortality of tomato.
Source: Authors

that the inoculum potential in the soil has decreased. It went from 107×10^6 to 411×10^5 CFU g^{-1} of dry soil. The reduction is of the order of $69\times10^6 \pm 669\times10^5$, that is an average reduction of 55.63 ± 27.8%. The analysis of the results (Figure 4) of the inoculum potential shows a very highly significant difference between the control and the other treatments (P = 0.0001).

Effects of different treatments on disease incidence in the field

Figure 5 shows the effects of treatments on disease incidence. The comparison of the means of the different treatments shows a reduction in tomato mortality of 41.0 ± 24.05%. There is a very highly significant difference between the treatments (P = 0.0001). The best sanitizing treatment is solarization with a reduction in tomato plant mortality of 60% compared to bare soil (34%). The other sanitizing treatments give statistically equal results.

The analysis of Figure 6 shows a very highly significant difference in the severity of the disease in tomato compared to sanitizing treatments (P= 0.0001). From the 22nd DAT (days after treatment) the disease appears in all treatments. The progression of the disease is remarkable (11% to more than 26%) in the plots having

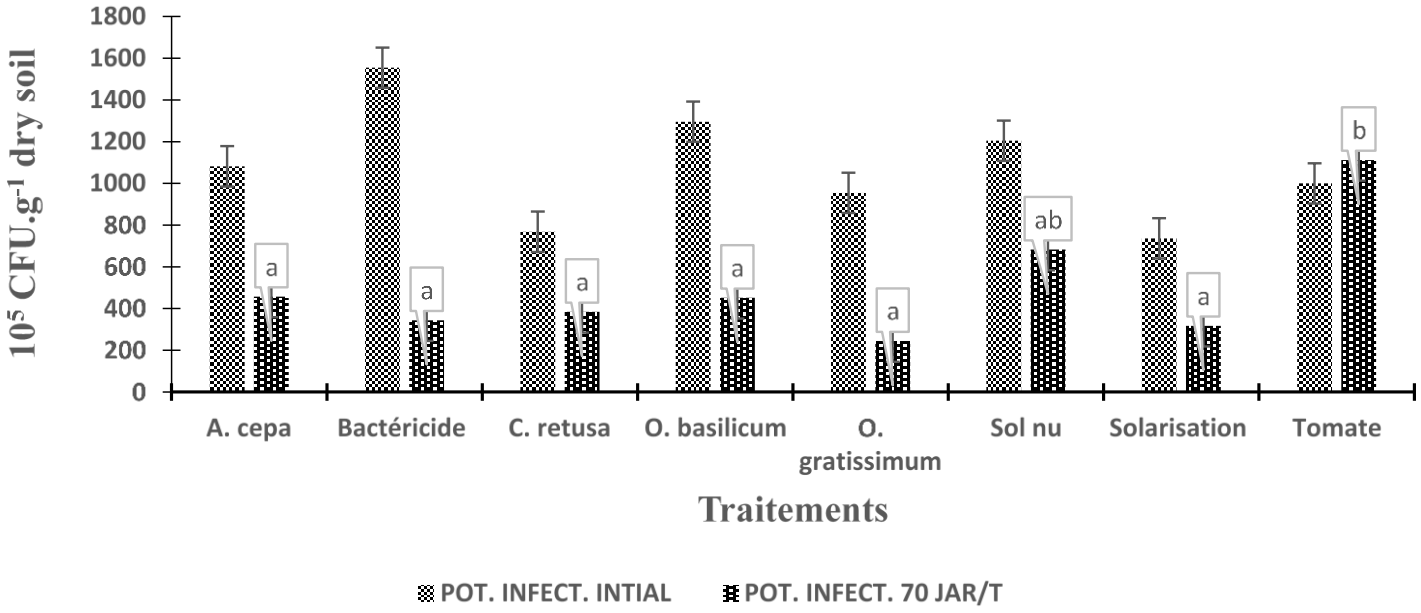

Figure 4. Effects of treatments on the inoculum potential of soil in the field. Numbers assigned the same letter do not differ significantly at the 5% threshold (Newman-Keuls Test).
Source: Authors

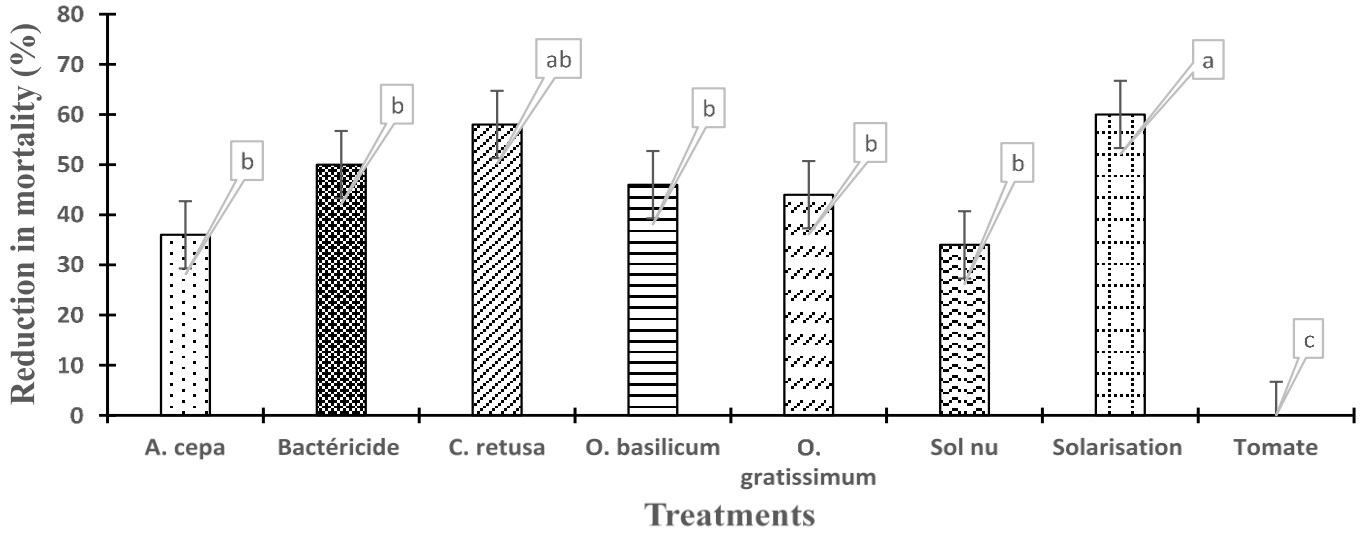

Figure 5. Effects of treatments on tomato mortality. Numbers assigned the same letter do not differ significantly at the 5% threshold (Newman-Keuls Test).
Source : Authors

received the tomato compared to the other treatments during the observations. In fact, solarization and *C. retusa* differ significantly from other treatments with an evolution of the disease between 4 and 10%.

DISCUSSION

The presence and the high rate of inoculum of *R. solanacearum* on the experimental site in Toussiana would be due to the monoculture of Solanaceae (tomato, eggplant, pepper, etc.). Indeed, the monoculture favors the conservation of the bacterium in the rhizosphere (Granada and Sequeira, 1983). A similar study showed that repeated monoculture of potato favored the multiplication of bacterial wilt in Niger (Adam, 1996). Also, the location of the site at the bottom of the slope could favor the drainage and the accumulation of the inoculum

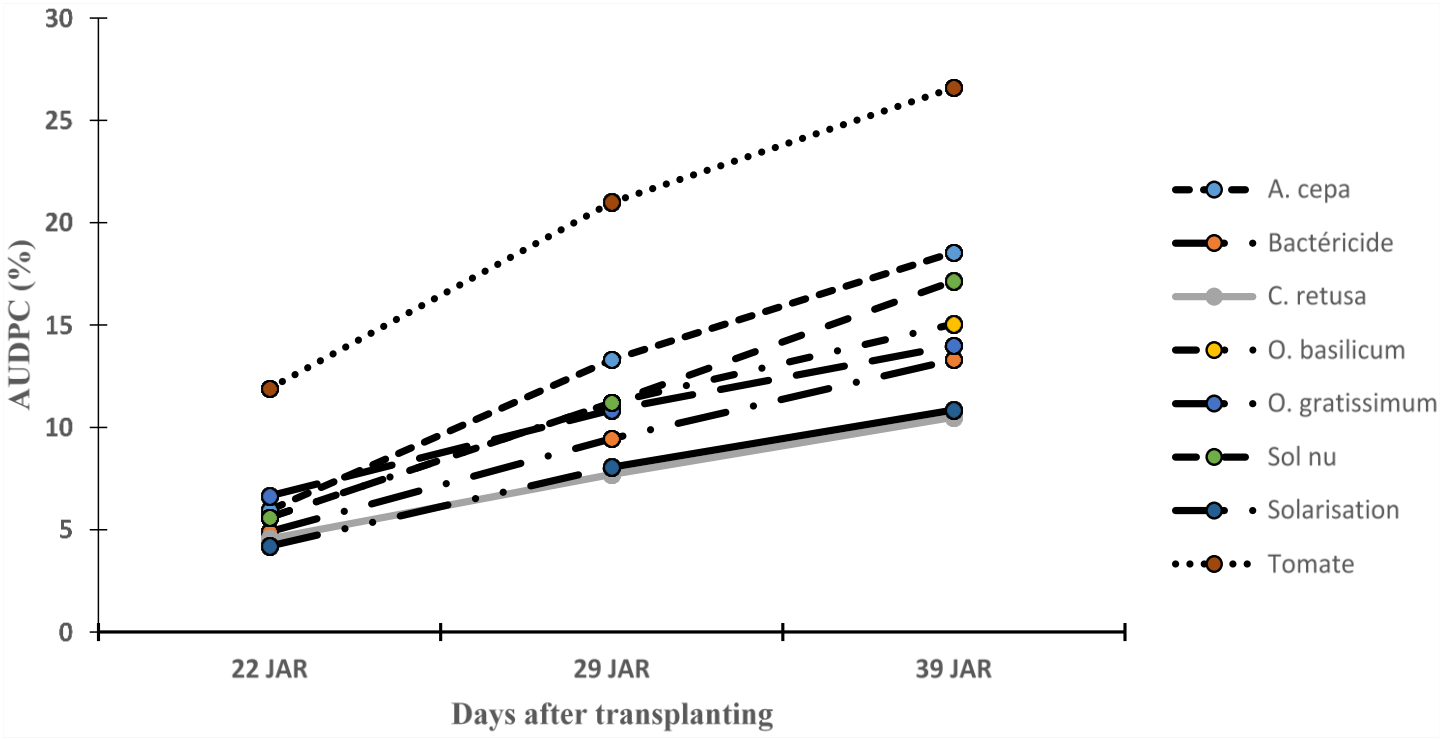

Figure 6. Disease progression over time.
Source: Authors

of the fields upstream on the test plot. This is what several authors indicate in their work, in particular Olsson (1976) and Farag et al. (1999). Furthermore, the proximity of the test site to the water course could create an environment favorable to the development of the disease. Thus, according to Kelman (1953) and Buddenhagen and Kelman (1964) the bacterium survives better in moist, well-drained soil than in dry or flooded soil and its optimum temperature for survival is between 30 and 35°C. In addition, soil of the site has a sandy dominance which is favorable to the preservation and development of the bacteria (He et al., 1983). The results obtained corroborate those of Somtoré (2017) who evaluated 1.37×10^5 CFU g^{-1} of dry soil as the average inoculum potential of the Yéguérosso market gardening site in the same province. In addition, the work of Somé (2001) and Nikiéma (2016) showed the presence of the bacterium in vegetable plots in Toussiana. The presence of phylotype I on the site would be due to exchanges of germplasms with neighboring countries such as Côte d'Ivoire where phylotype I is found. Thus the transport and use of infested plant material could be the cause of the dissemination of *R. solanacearum* (Hayward, 1991). Moreover, the importation of latently infested potato tubers is believed to be the cause of outbreaks of bacterial wilt declared in Europe (Digat and Caffier, 1996). This strong presence of phylotype I is in accordance with the work of Ouédraogo (1998) who also reported the presence of phylotype I (Race 1, biovar III and IV) in Burkina Faso. Similarly, Théra et al. (2010) had come to the same result in Mali on potatoes, as well as N'Guessan et al. (2012) in Ivory Coast. The high incidence of the disease in the trial in just four (4) weeks after transplanting, 56 DAS, would be explained by the virulence of phylotype I (Traoré et al., 2018) and the large amount of inoculum in the soil, in the sense that the optimum threshold for inducing the disease is 10^8 CFU.g^{-1} of dry soil (Winstead and Kelman, 1952). Similarly, it is noted that the cv. Rossol is sensitive to bacterial wilt (Somtoré, 2017). Moreover, environmental factors (temperature, sunshine) strongly influence the incidence of the disease (Buddenhagen and Elsasser, 1962). The results obtained corroborate those of Somtoré (2017) who evaluated 1.37×10^5 CFU g^{-1} of dry soil; the average infectious potential of the Yéguérosso market gardening site in the same province. The decrease in the quantity of soil inoculum and the reduction in the incidence of the disease by the onion would be due to the fact that it is not a host of *R. solanacearum* (Groshens, 2009; Deberdt et al., 2012). In addition, the root emission of thiosulfinate and the strong mycorrhization of the onion do not favor the development of the bacteria (Fernandes et al., 2012). This reducing effect attributed to the emission of mixed thiosulfinate by the roots has been successfully demonstrated during rotations and tomato associations with *Allium* (Yu, 1999). Aqueous extracts of *Allium*

fistulosum also showed strong sanitizing power on *R. solanacearum* in natural soil (Groshens, 2009). The same effects observed in *C. retusa* could be explained by the combination of several factors. *C. retusa* produces exudates (pyrrolizidine), which have a biocidal effect on *R solanacearum* (Fernandes et al., 2012, Damien, 2013). Moreover, the high frequency of mycorrhization (3-12 times more than tomato) and nodulation, which promotes nitrogen nutrition in *C. retusa*, could promote the multiplication of microorganisms antagonistic to *R. solanacearum*. Antagonism creates competition for the colonization of nutrient sites (Zhu and Yao, 2004, Fernandes et al., 2012). The nitrogenous nutrition of tomato stimulates its defense mechanism, hence the reduction in the incidence of the disease (Fernandes et al., 2012). These results are consistent with previous work by Fernandes et al. (2012) and Damien (2013), with the use of *C. spectabilis*.

The remarkable effect of solarization is likely linked to the fact that the transparent plastic film creates a high temperature (> 43°C), which makes it possible to reduce the quantity of bacteria in the soil; and therefore the incidence of the disease is reduced (Gamliel et al., 2000). The strong increase observed in the inoculum potential in semi-controlled environment compared to the field would be linked to the late inoculation which allowed the tomato plants to reach the fruiting stage with the disease and remained in the pots until the sampling period (Hayward, 1991).

Conclusion

The aim of this study was to reduce the incidence of bacterial wilt caused by *R. solanacearum* through the process of sanitation of the plants. The evaluation of the inoculum potential showed that the study site is infected with an average of 1.07×10^8 CFU g^{-1} of soil. The various treatments reduced the inoculum potential of the soil in *R. solanacearum* by 68.22% in a semi-controlled environment and by 55.63% in the open field. The incidence of the disease fell by 41% in the open field. The best sanitizing plant, in a semi-controlled environment, is *C. retusa* (73.96%), and *O. basilicum* (66.8%). In the field, *O. gratissimum* is the best sanitizing plant with 68.18% reduction in inoculum potential. The disease manifests less with *C. retusa* in the field. Bare soil registers the lowest reduction at all levels. The monoculture of tomato increases the infectious potential of the soil and the incidence of the disease. The sanitizing plants are an alternative for fighting against this disease.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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