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African Journal of Plant Science

Full Length Research Paper

Prospecting, survey and plant material collection for *Hibiscus sabdariffa* L. (Malvaceae) genebank establishment in Côte d'Ivoire

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***Hibiscus sabdariffa* L. (Malvaceae) is a plant whose leaves and calyxes are integral to the local diet. The leaves are commonly used in sauce preparations, while the calyxes are utilized in making the local drink known as "bissap," which is highly appreciated by the local population. Despite its socio-economic significance, the production of this species remains relatively low in Côte d'Ivoire. This decline in yield may be attributed to the lack of available information regarding the identification of different *H. sabdariffa* cultivars. To establish a comprehensive database, a survey and collection of various local cultivars of *H. sabdariffa* were conducted in 12 locations across Côte d'Ivoire. In total, 80 accessions were recorded, with 53.75% of them located in the North. Among these collected accessions, two primary cultivar types were identified based on calyx coloration: the red type (Hibiscus sabdariffa var sabdariffa) and the green type (*Hibiscus sabdariffa var altissima*). Within these two types, the presence of other morphotypes has been observed. Cultivation and trade of these cultivars are primarily carried out by women, with over 90% of them having no formal education. The price of calyxes of the red type varies from 3000 to 4700 FCFA per kg, providing an opportunity for farmers to diversify their sources of income. The various accessions collected thus constitute a substantial genetic reservoir necessary for the development of new *H. sabdariffa* varieties.**

Key words: *Hibiscus sabdariffa*, *ex-situ,* Collection, Seed genebank, Côte d'Ivoire.

INTRODUCTION

Countries need genetic resources to sustainably increase their agricultural production. This is particularly important for countries which are south of the Sahara, where agriculture is the main currency sources. Also, in the face

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of rapid population growth, crop diversification is essential to adapt to the adverse effects of climate change and to meet the challenges of the food crisis. The need and urgency to ensure food security and economic prosperity for the populations of developing countries today necessarily involves the enhancement and promotion of crops of local interest (Sourabie et al., 2020).

Indeed, local crop plants abound in traits of interest, notably adaptation and resistance to biotic and abiotic pressures (Garcia et al., 2021). Such plant species are generally found in developing countries, where the introduction of cash crops to improve farmers' incomes and living standards has led to their abandonment. As a result, local plant species of less immediate economic interest are rapidly declining in traditional production systems.

One example is Roselle, *Hibiscus sabdariffa* L., a traditional leafy vegetable whose flower calyx is also used to prepare tea and the widely consumed local beverage (bissap) (Ross, 2003). *H. sabdariffa* is also used to regulate blood pressure and to treat liver ailments and fever (Wang et al., 2000). *H. sabdariffa* is an antioxidant, anticancer agent and contains organic acids and other vitamin compounds (Salah et al., 2002). It is rich in polyphenols and vitamin C (Fall, 2001). Anthocyanins extracted from Roselle callus may have a protective effect against atherogenesis through their antioxidant capacity (Abeda et al., 2015).

However, despite its socio-economic importance, *H. sabdariffa* production remains relatively low (Cissé et al., 2008, Alassi et al., 2017). Improving *H. sabdariffa* production, which is well adopted by farmers, could be beneficial for the rural communities. To obtain improved varieties that are both productive and stable, the breeders must have at his disposal the greatest possible genetic diversity of the species studied (Baudoin, 2001). Prospecting missions, including surveys and collections of *H. sabdariffa* accessions in seed form, have therefore been carried out in Senegal (Diouf et al., 2004), Benin (Alassi et al., 2017) and Burkina Faso (Ouangraoua et al., 2021). The aim was to establish a genebank and obtain genetic data on local cultivars of interest for the direct development of the species at national level.

In Côte d'Ivoire, little information is available on the morphological, agronomic, biochemical and molecular characteristics of *H. sabdariffa* cultivars found in village land. The research activities about regeneration from cuttings (Sié et al., 2008) and agromorphological characterization (Sié et al., 2009) have been carried out using accessions obtained in only two localities. Although the species is grown in several Côte d'Ivoire agro-climates, only *H. sabdariffa* seeds were collected from various markets in Abidjan (South) and Korhogo (North) (Sié et al., 2008,2009). Previous research was also undertaken on the two forms *Hisbiscus sabdariffa* var *sabdarrifa* and *Hisbiscus sabdariffa* var *altissima* from Korhogo locality alone, to demonstrate the effect of explant genotype and culture medium composition on *in vitro* regeneration via somatic embryogenesis (Sié et al., 2010). In 2011, seeds collected in the Korhogo locality were used to reveal the *in vitro* resistance of the sabdariffa form and the sensitivity of the altissima form to one of the species' major pests, the fungus *Fusarium oxysporum* (Boulanger et al., 1984).

A collection representative of the diversity of *H. sabdariffa* in Côte d'Ivoire would be essential to guarantee the sufficiency of genetic data and to establish a breeding programme to improve this species for the benefit of rural communities. Knowledge about the various local cultivars of *H. sabdariffa* first requires an inventory of existing cultivars, to provide the breeder with a varied range of genotypes within the two forms already reported. This study was carried out in order to establishing an ex situ genebank representative of the diversity of *H. sabdariffa* from existing local diversity in Côte d'Ivoire.

MATERIALS AND METHODS

Plant material and study area

Surveys and collections of *Hibiscus sabdariffa* seeds were carried in 12 localities in Côte d'Ivoire from September 2022 to March 2023. Of these, 05 were in the North (Ferkessédougou, Boundiali, Korhogo, Kanawolo and Niaka), 03 in the West (Bangolo, Daloa and Duekoué), 02 in the East (Bondoukou and Agnibilékro), 01 in the center (Bouaké) and 01 in the South (Abidjan) of Côte d'Ivoire. These locations were selected to cover all the country's agro-ecological zones. The localities surveyed are shown in Figure 1.

METHODS

In each locality, the participatory method exploiting the endogenous knowledges of the famers (producers and traders) both men and women as described by Adoukonou-Sagbadja et al. (2006) and Dansi et al. (2010) was used to obtain information (Figure 2). The data collection method consisted of direct interviews with *H. sabdariffa* farmers and traders randomly selected in the localities surveyed. This survey method was based on a list of questions drawn up in advance. The questionnaire was designed with the survey's objectives in mind. It concerns the identification of farmers and traders, the origin of cultivated seeds, farming practice, method of obtaining and preserving seeds, criteria for identifying local cultivars and economic data on *H. sabdariffa*.

Samples of *H. sabdariffa* seeds were collected both from markets in the various localities visited, in the field and directly from the seed stocks of the famers surveyed, where available. The seeds of the various cultivars collected were then placed in labelled envelopes to be entered as accessions in the collection under a unique 7-digit alphanumeric code. Accessions are coded using the first two letters of the species' scientific name (HS), followed by two letters indicating the abbreviated name of the locality and three arabic numerals indicating the chronological order of introduction into the genebank. For example, the coded accession HSDA001 represents the first accession (001) of *H. sabdariffa* (HS) introduced

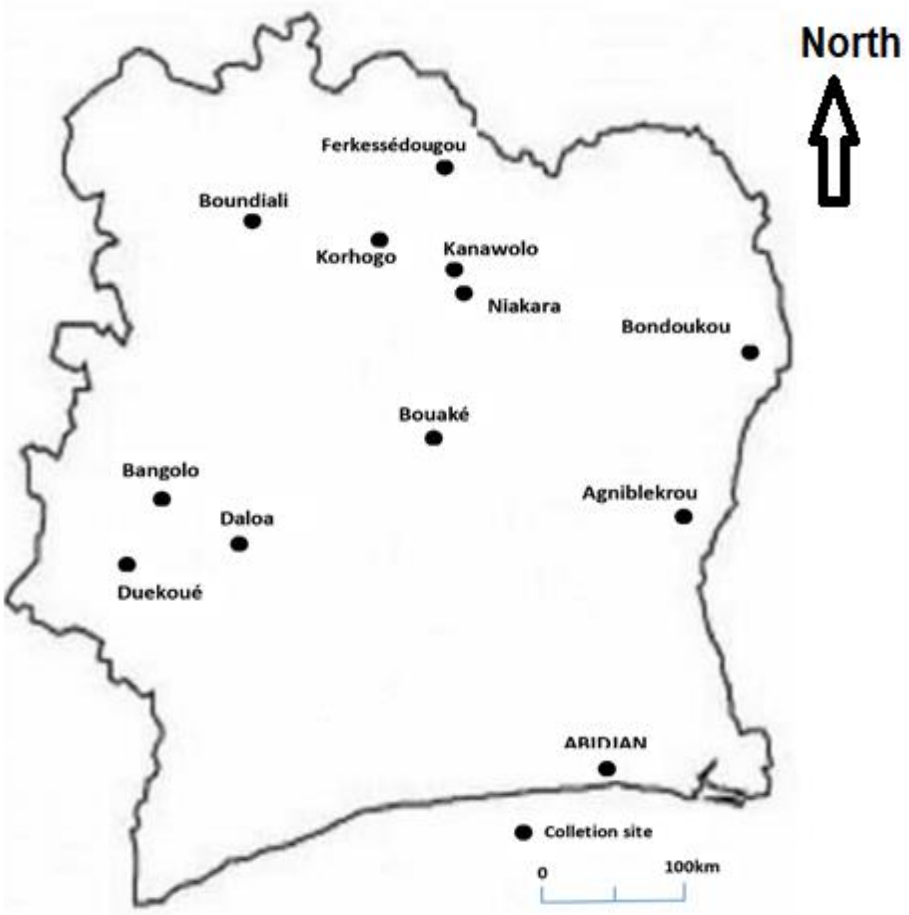

Figure 1. Map of Côte d'Ivoire with surveyed localities.

Figure 2. Interview by the research team (a) directly with the producer; (b) in a group with women producing *Hibiscus sabdariffa* in the village of Tchepke in the Ferkessédougou locality.

Table 1. Number of accessions collected according to localities and introduced into three genebanks in Côte d'Ivoire.

Zone	Localities	Accessions numbers	Gene Bank of UPGC	Gene Bank of UJLoG	Gene Bank of UNA
North	Korhogo	20	x	x	x
	Ferkéssedougou	07	x	x	x
	Boundiali	04	x	x	x
	Niakara	09	x	x	x
	Kanawolo	03	x	x	x
Centre	Bouaké	07	x	x	x
West	Duékoué	02	x	x	x
	Bangolo	03	x	x	x
	Daloa	01	x	x	x
Est	Bondoukou	01	x	x	x
	Agnibilékrou	03	x	x	x
South	Abidjan	20	x	x	x
Total	12	80	x	x	x

UPGC: University of Peleforo GON COULIBALY; UJLoG: University of Jean Lorougnon Guédé; UNA: University of Nangui Abrogoua; x: Presence of the accession in the genebank of the research institution.

into the seed genebank from Daloa (DA) locality.

Statistical analyses

Prospecting, survey and collection data were subjected to descriptive analysis (frequency, mean, percentage) using R software version 4.1.2 (R Core Team, 2021). The results are presented in tabular and graphical form.

RESULTS

Valuation of accessions sampled at locations visited

Eighty (80) *Hibiscus sabdariffa* accessions were collected in all the localities visited. Of these, 43 (53.75%) were found in the North. Twenty accessions (25%) came from in the South. In the Centre, 7 (8.75% of accessions) were counted. Six (7.5%) and 4 (5%) accessions came from Western and Eastern Côte d'Ivoire respectively (Table 1). Of all the accessions collected, two types can be distinguished: the red type was abundantly reported (67.50%) compared with the green type (33.50%).

To optimize the strategy of conserving *H. sabdariffa* accessions in Côte d'Ivoire, the genebank was duplicated in three replicate and hosted by the University of Peleforo GON COULIBALY (UPGC) at Korhogo, the University of Jean Lorougnon Guedé (UJLoG) at Daloa and the University of Nangui Abrogoua (UNA) at Abidjan (Table 1). The seed genebank for *H. sabdariffa* at the UPGC (Korhogo) is hosted by the National Shea Research Program, which aims to promote crops associated with shea parklands in order to enable rural communities to adapt to the adverse effects of climate change by diversifying their crops. All accessions were surveyed from 205 persons (men and women), including 151 traders and 54 farmers. More than half of those surveyed, 58.20%, were from the North. Next come the West and South with 19.40 and 11.95% respectively. The two other zones, the Centre and the East, had the lowest numbers of person surveyed, with 6.47 and 3.98% respectively.

Characteristics of the people surveyed

The people surveyed comprised 15 men (7.31%) and 190 women (92.69%). In terms of traders, *Hibiscus sabdariffa* is sold mainly by women (90.72%). The age of the traders surveyed ranged from 20-30 years to 50-60 years. The 30-40 age group is in the majority with 51.65%, followed by the 40-50 age group with 26.50%. The other two age groups, 20-30 and 50-60, account for 13.90 and 7.95% respectively. Cultivation is also practiced almost exclusively by women, with 97.15%. Among these farmers, 83.3% have no formal education. This population is subdivided into three groups according to age. The 20-30 age group, comprise 92.3% of farmers

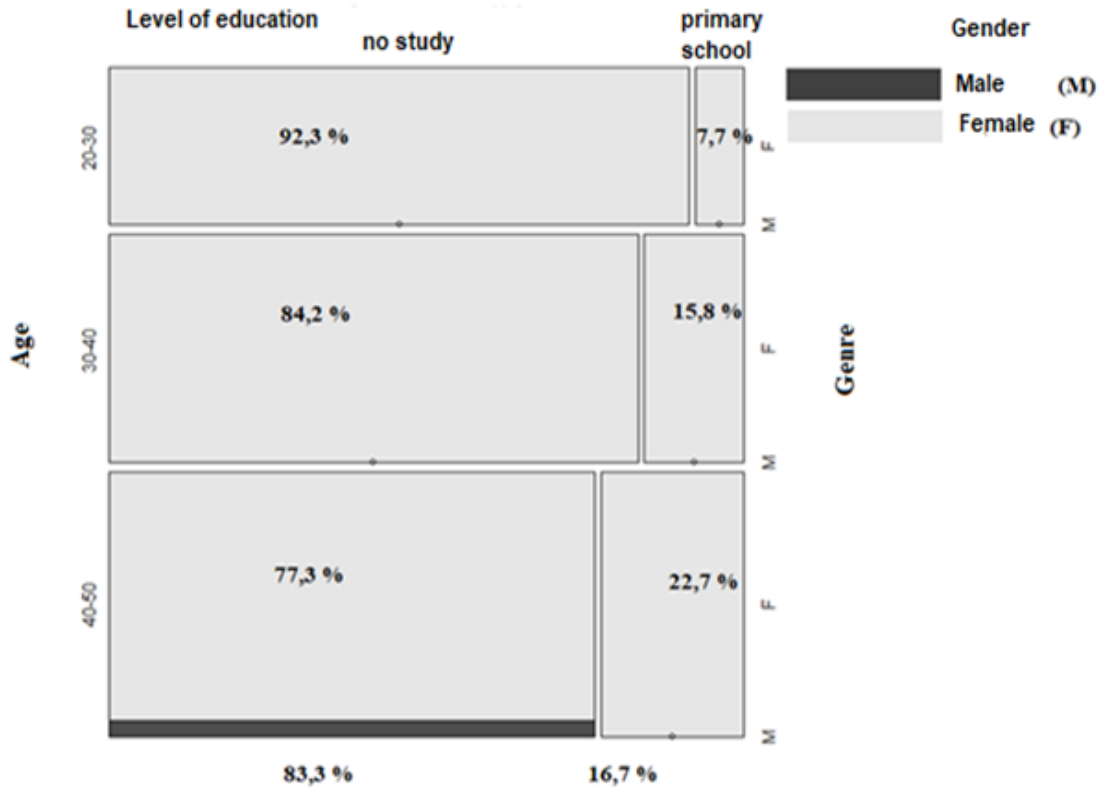

Figure 3. Characteristics of farmers by age, level of education and gender.

with no schooling. The 30-40 and 40-50 age groups have 84.2% and 77.3% respectively of farmers with no schooling (Figure 3).

Methods of supply and origins of *Hibiscus sabdariffa* seeds grown or sold

A proportion of 90.74% of grower surveyed reported that they acquired seeds through previous cultivation, while 9.26% acquired seeds by purchase. In the case of traders, 13.90% acquire seed through their own production and 86.10% through purchase. The origin of seeds comes from Burkina with 49%. 41.07% of seeds come from Korhogo, Ferkessédougou and Boundiali, (with 17.22%, 16.55% and 7.30% respectively). Duekoué and Mali account for 3.97 and 3.30% respectively. The other towns, notably Séguela, Bonoufla, Katiola and Napié, represent 0.66% each.

Description and use of *Hibiscus sabdariffa*

People surveyed in Northern, Central and Eastern Côte d'Ivoire recognized the existence of two cultivars based on calyx color within *Hibiscus sabdariffa*: the green cultivar (*Hibiscus sabdariffa* var. *altissima*) and the red cultivar (*Hibiscus sabdariffa* var. *sabdariffa*). Proportions of 33.33% and 17.50% of People surveyed in the South and West respectively recognize only one cultivar, the red one. According to information received from traders in the Centre and North, the leaves of both cultivars are eaten in sauces. In other areas, the red-calyx cultivar is used exclusively to produce the fresh juice widely known as "bissap", while the leaves of the green-calyx cultivar are used in sauces. Accessions collected from traders and growers show that it is difficult to distinguish between the seeds of the two cultivars (Figure 4a and b). On the other hand, the calyxes of the red cultivar sold on the various markets are of two types: light red and dark red. The dark-red calyx comes from Burkina Faso and is more popular with traders because of its high demand. Within the green type, two morphotypes according to leaf size have been reported: the large-leaf morphotype and the small-leaf morphotype (Figure 4c and d).

Survey work revealed that in the Senoufo language, the red morphotype is called *Tangnin* and the green type, *Tanga*. In the Malinké language, *Dawoueni* is the name of the red morphotype and *Dah*, the green morphotype. In almost all ethnic groups, *Hibiscus sabdariffa* is

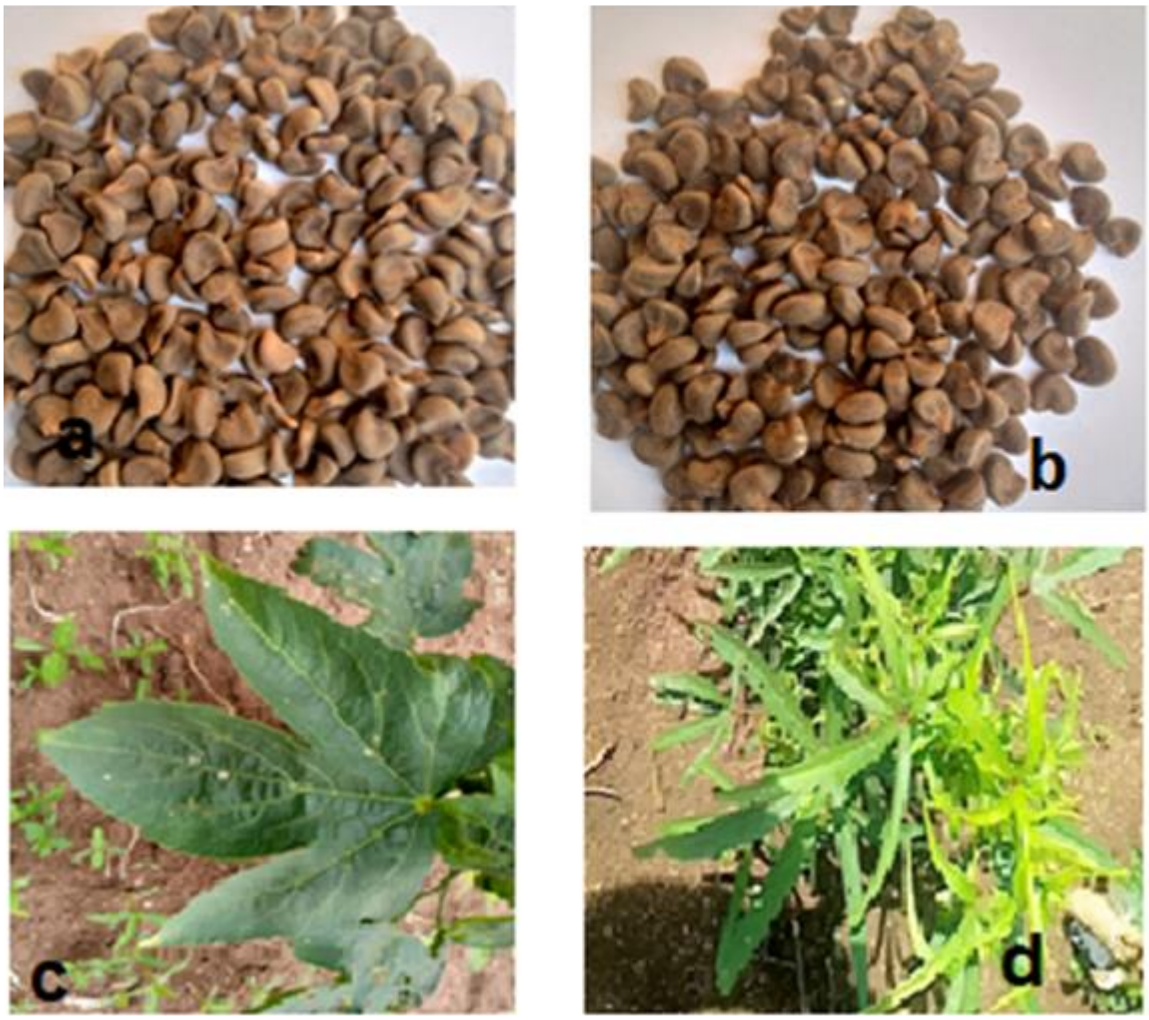

Figure 4. *Hibiscus sabdariffa* seeds and leaves; a: Seeds of the red calyx cultivar; b: Seeds of the green calyx cultivar; c: big leaf of the green calyx cultivar; d: small leaf of the green calyx cultivar.

commonly known as bissap. As a result, its vernacular names are ignored or unknown.

Constraints to *Hibiscus sabdariffa* production in Côte d'Ivoire

Constraints to *Hibiscus sabdariffa* production are generally linked to disease and pest damage to leaves and flowers. According to traders, in 2022, the plants were severely attacked by jassids (Homoptera: Cicadellidae), resulting in considerable production losses. Control methods need to be deployed against these pests, which pose a serious threat to the survival of *H. sabdariffa* cultivation in Côte d'Ivoire. Growers do not resort to the use of phytosanitary products for lack of financial means. Of all the growers surveyed, only 11.11% use pesticides chemical products, especially on plots used for market gardening. All the people surveyed had no knowledge of improved *H. sabdariffa* varieties, and polyculture was the only cultivation technique applied in all the plots visited. In central Côte d'Ivoire, *H. sabdariffa* is grown with cabbage, lettuce, tomato, okra and other crops. In the north, on the other hand, *H. sabdariffa* is grown with rice, groundnuts, cotton, cashew, maize, often in shea parks, home gardens, etc. Sowing begins at the start of the rainy season, practically in June. *H. sabdariffa* leaves are harvested two months after sowing for consumption or sale. The calyxes are also harvested during flowering, i.e. 3 to 4 months after sowing. Once harvested, the seeds are dried in the sun and stored in a canary until the next season. At the market, traders store seeds in bags (54.30%), plastic sachets (22.52%), cans (17.22%) and basins (5.96%),

Figure 5. Measuring instrument for sale in the market of *Hibiscus sabdariffa. (*a) Fresh leaves; (b) Tomato box for sale.

without the use of phytosanitary products against pests.

Some economic aspects of *Hibiscus sabdariffa* cultivation in Côte d'Ivoire

The price of *Hibiscus sabdariffa* seeds, leaves and calyxes varies according to the sales period and year, as well as availability. Fresh leaves are sold in heaps of 25 to 100 FCFA in the markets (Figure 5a). Dried calyxes of the red calyx cultivar are sold in large tomato cans (around 0.5kg of dried calyxes) (Figure 5b) and the selling price varies from 3,000 to 4,700 FCFA per Kg, depending on availability. In the dry season, the quantity of dried calyxes on the market drops, so the price is high. The price of seeds of both types is relatively identical, ranging from 750 to 1000 FCFA per Kg.

DISCUSSION

The overall aim of this study is to improve the production of *Hibiscus sabdariffa* and socio-economic situation of rural populations in Côte d'Ivoire. Survey of local cultivars is a prerequisite for any genetic improvement strategy for this species. A total of 80 accessions were collected in the main agro-ecological zones of Côte d'Ivoire. Of these, 53.75% were found in the North. In this zone, *H. sabdariffa* leaves are widely consumed in sauces, and have long been an integral part of the population's dietary habits. Cultivation of this species is more suited to the North, which remains the epicenter of *H. sabdariffa* cultivation in Côte d'Ivoire. Nevertheless, almost half of all *H. sabdariffa* seeds grown during this survey originated in Burkina Faso. Indeed, the Western part of Burkina Faso, which borders northern Côte d'Ivoire, is the leading area for *Hibiscus sabdariffa* production (Ouangraoua et al., 2021). Northern Côte d'Ivoire is therefore a zone of massive and regular importation of this species.

Over 90% of *H. sabdariffa* cultivation and trade is carried out by women. Indeed, in Africa, the cultivation of traditional leafy vegetables is an activity essentially reserved for women (Diouf et al., 1999). In our studies, we found that over 80% of women interested in growing *H. sabdariffa* had no formal education. The schooling of young girls should be encouraged. This will enable them to easily master the cultivation techniques for this species, with a view to improving production.

Two types of *H. sabdariffa* have been reported. These are the red type and the green type. Within the red type, two cultivars, dark red and light red, were recorded during the surveys. These numbers are lower than those recorded in Senegal by Cissé et al. (2008), who counted seven cultivars, only three of which originated in Senegal. The low diversity found within this species may be due to its predominantly autogamous mode of reproduction, which is not conducive to genetic cross-fertilization. According to the testimonies gathered, the dark red cultivar is the most appreciated for its high acidity and the quantity of bissap juice it yields. Leaves of the red variety are also eaten in sauces, as are leaves of the green variety. This latter was found in almost all the markets we visited, but the large-leaf cultivar is more in demand than the small-leaf variety. It has also been reported that women leaf growers in Senegal prefer the large-leaf green cultivars (Diouf et al., 2004). From a nutritional point of view, *H. sabdariffa* leaves are very rich and could make a significant contribution to improving human nutrition (Soro et al., 2022). While the seeds of *H. sabdariffa* are much sought-after and used in food in Mali for their richness in amino acids and fatty acids (Fane et al., 2021), most growers we met in Côte d'Ivoire do not harvest the seeds. Leaves and calyxes are harvested at the vegetative and flowering stages respectively. The pods are left in the field. They burst at some point under the effect of the sun, releasing the seeds which germinate in the next rainy season.

Various field visits have shown that farmers grow *H.*

sabdariffa in association with other crops. This is because it is not considered a main crop. This combination of crops is thought to be one of the reasons for the low production of *H. sabdariffa*. This is confirmed by the research work of Faye et al. (2001), which revealed that *H. sabdariffa* yields are lower in association with cereals, notably millet and sorghum. In addition, insect pests destroy the leaves and flowers. During our surveys, only 11.11% of farmers used phytosanitary products. Lack of financial means is one of the reasons cited. However, for soil fertility, some farmers in Benin have had the merit of using animal excrement, notably cattle and poultry, to increase *H. sabdariffa* production (Alassi et al., 2017). At market level, the storage of seeds in makeshift objects such as cans, bags and plastic sachets allows pests to affect seed quality. Although *H. sabdariffa* is considered a minor crop according to Cissé et al. (2008), it is currently a significant source of income for farmers. Farmers grow it for their own consumption, and the surplus is sold on markets. The relatively more profitable price of red morphotype calyxes, ranging from 3,000 to 4,700 FCFA per kg, would be an opportunity for farmers to diversify their sources of income.

Conclusion

The aim of the present study was to establish an ex-situ genebank representative of the diversity of *H. sabdariffa* from existing local diversity in Côte d'Ivoire. Accessions were collected in the main agro-ecological zones of Côte d'Ivoire, resulting in the identification of 80 accessions, over 50% of which come from the north. Two cultivars were reported: the green cultivar and the red cultivar. The existence of morphotypes within these two types was also reported by the people surveyed. These cultivars are mainly grown and traded by women. The plant is grown in association with other crops, notably rice, millet and groundnuts. Morphological and molecular characterization studies should be carried out to gain a better understanding of the performance of *H sabdariffa* accessions and facilitate their use in varietal selection programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

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:

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

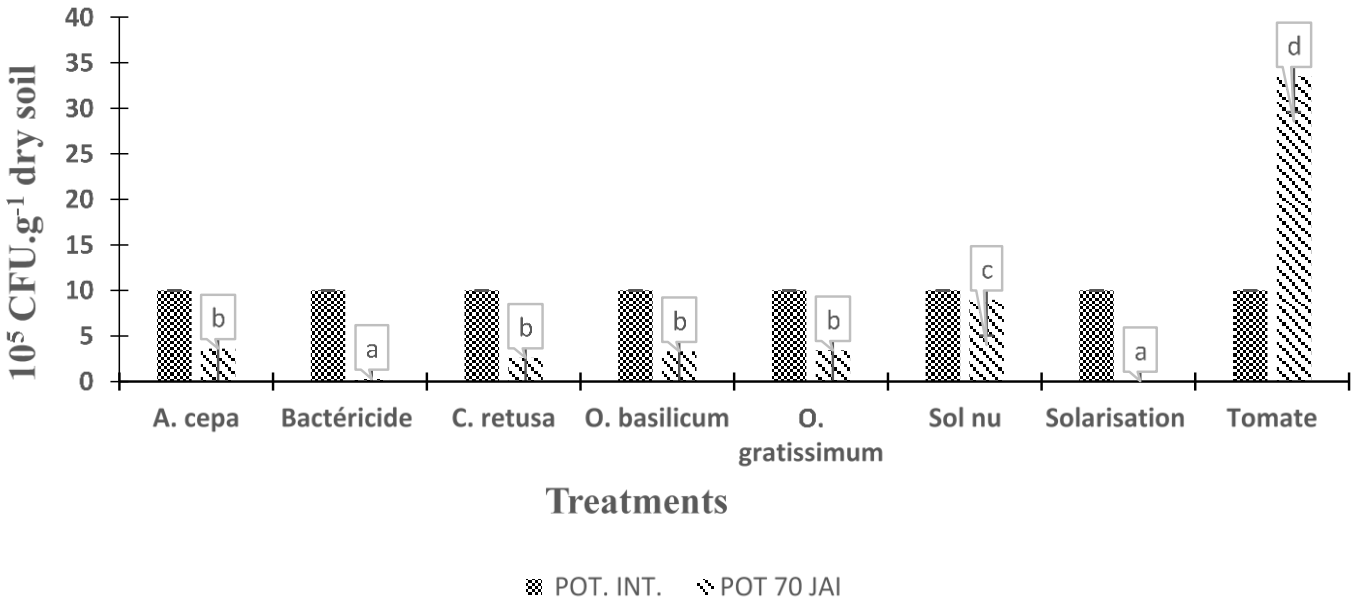

Figure 1. Effect of treatments on the inoculum potential of the soil in a semi-controlled environment. Column numbers assigned the same letter do not differ significantly at the 5% threshold (Newman-Keuls Test).
Source: Authors

∑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

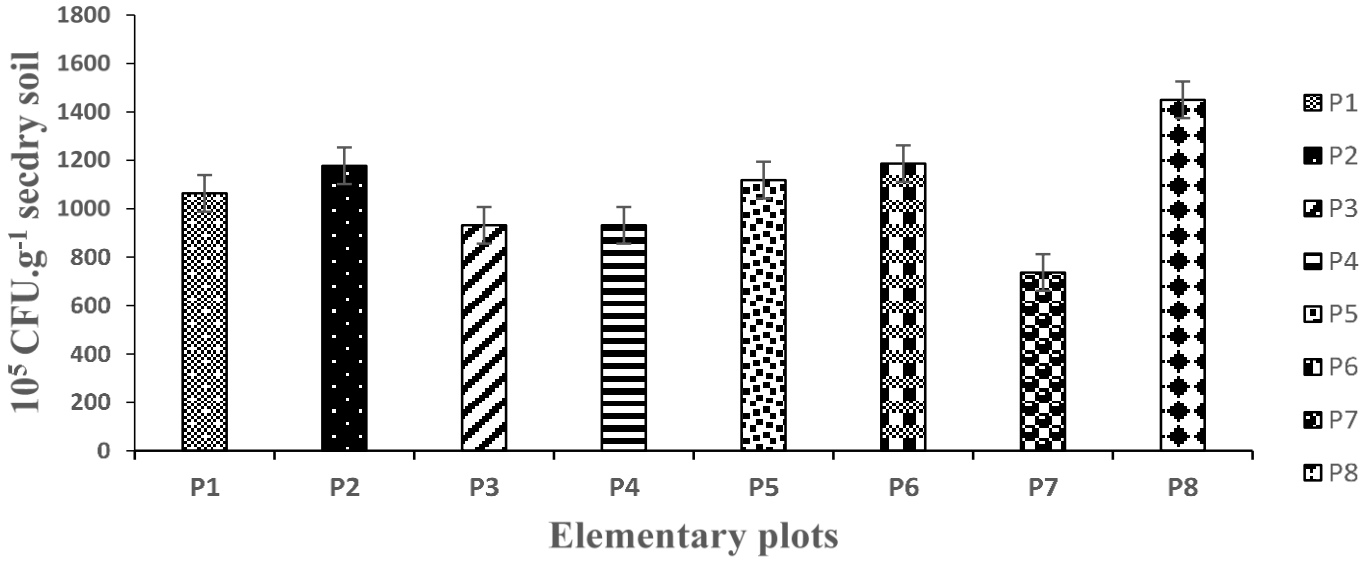

Figure 2. Initial inoculum potential of the experimental soil.
Source: Authors

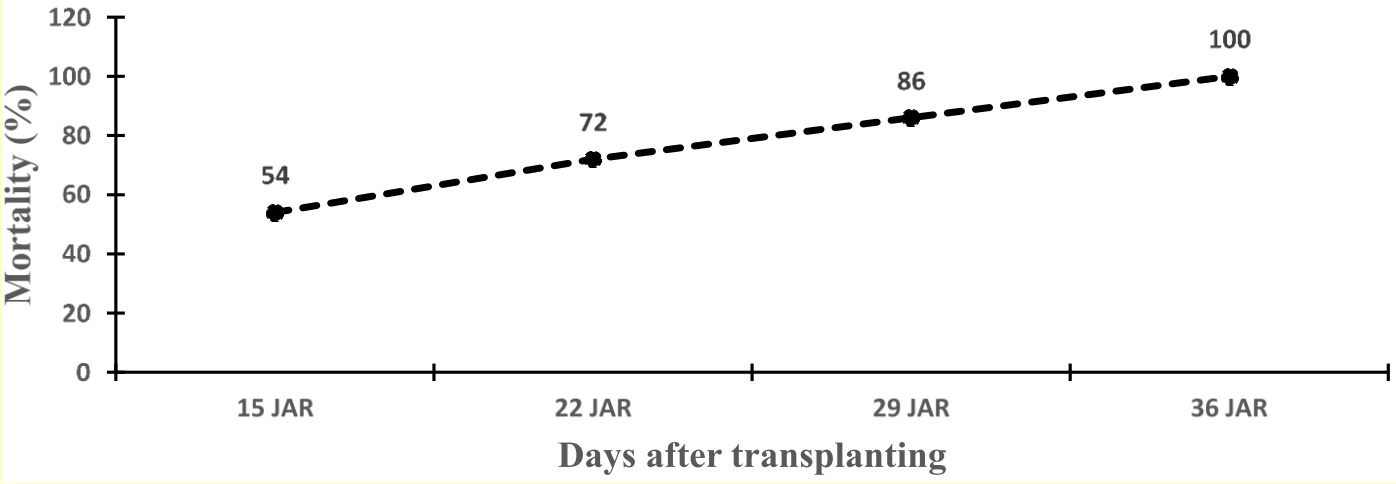

Figure 3. Progression of cumulative mortality of tomato.
Source: Authors

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

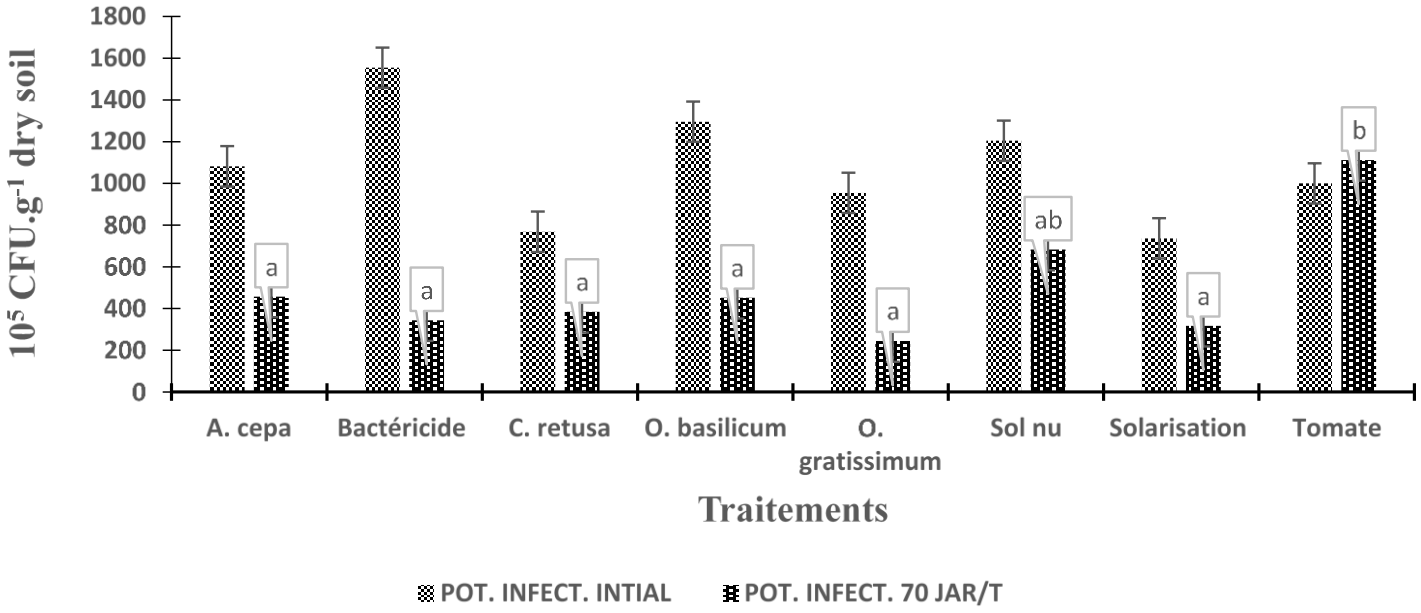

Figure 4. Effects of treatments on the inoculum potential of soil in the field. Numbers assigned the same letter do not differ significantly at the 5% threshold (Newman-Keuls Test).
Source: Authors

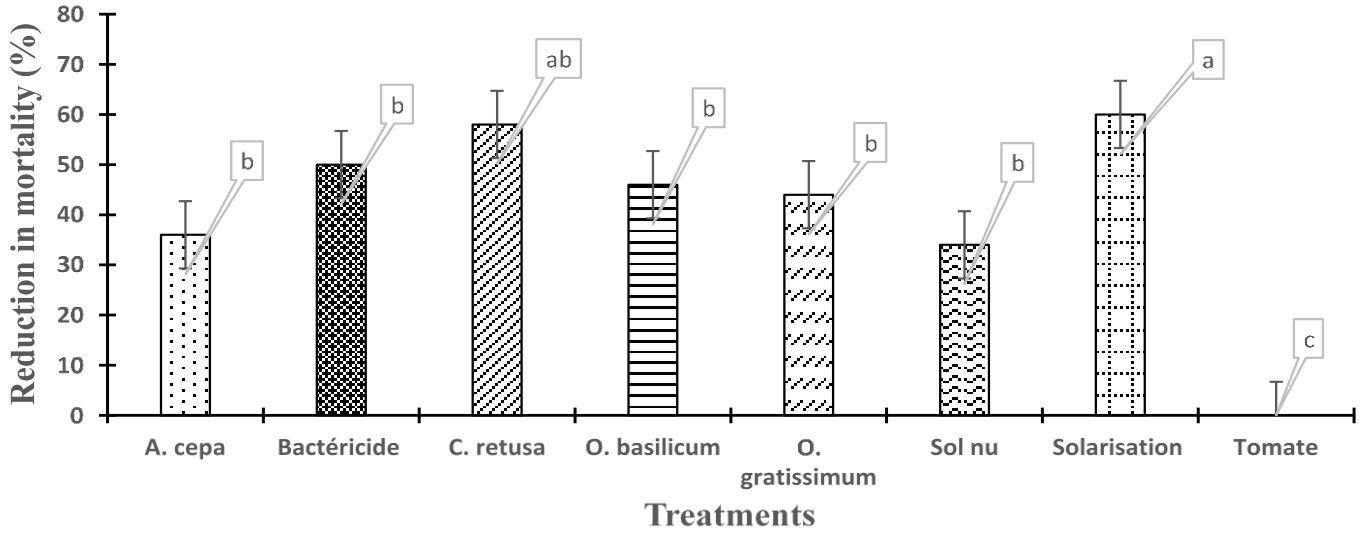

Figure 5. Effects of treatments on tomato mortality. Numbers assigned the same letter do not differ significantly at the 5% threshold (Newman-Keuls Test).
Source : Authors

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

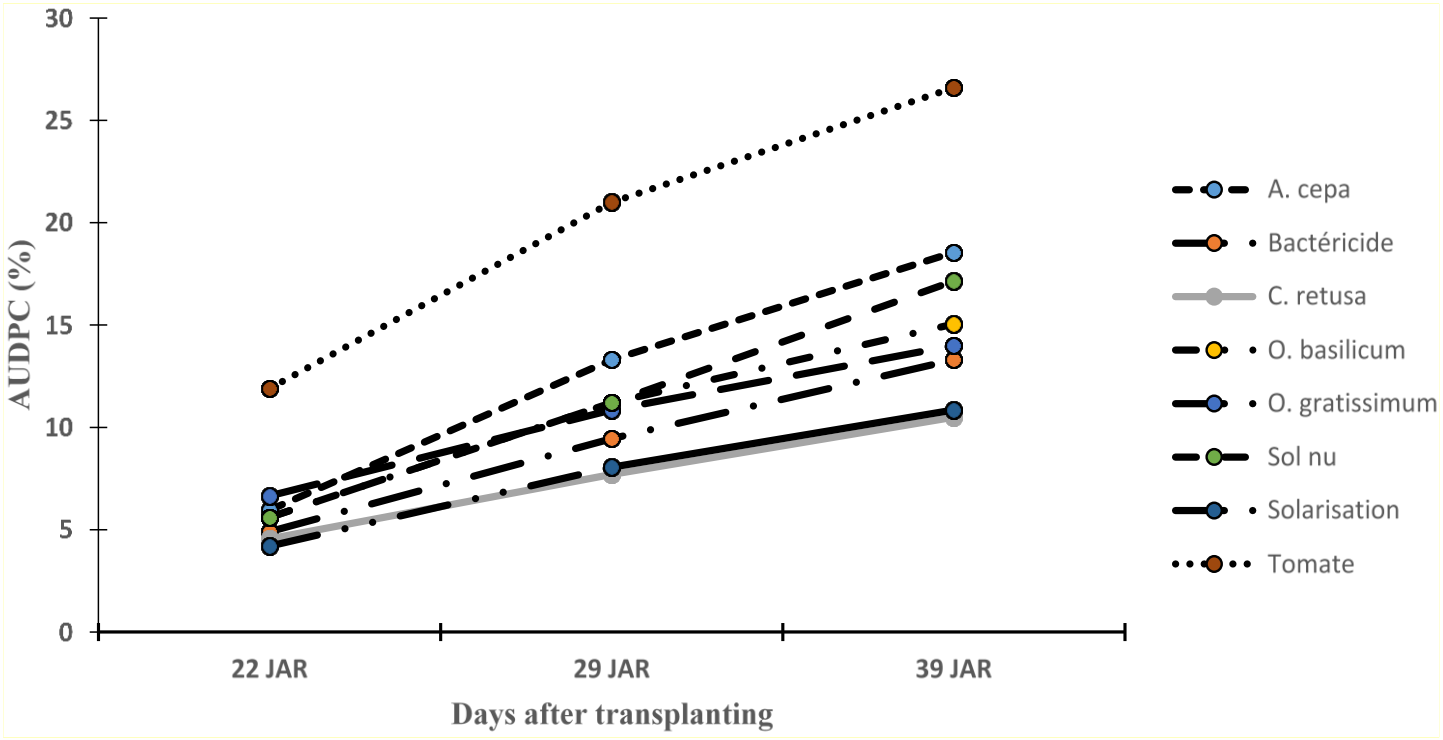

Figure 6. Disease progression over time.
Source: Authors

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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