

Efficacy of *Bacillus* spp. / isolates for application as biofertilizer in tomato (*Solanum lycopersicum* L.) cultivation.

ABSTRACT

Certain species of *Bacillus* are considered rhizobacteria that promote plant growth, inhibit the growth of plant pathogens and deleterious rhizospheric microorganisms. The aim of this study was to determine the stimulatory effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on the growth and disease reduction of tomato plants in Côte d'Ivoire. In this respect, three substrates consisting of soil only, sawdust only and a mixture of soil and sawdust were inoculated with the two bacteria, then left to ferment for twenty-four (24) hours. The biofertilizers obtained after fermentation were used to assess their effects on the development of potted tomatoes. Growth parameters, infection rate and mortality rate of tomato plants were measured regularly during 6 weeks of cultivation. A search for tomato pathogenic fungi was carried out to demonstrate the efficacy of *Bacillus* strains isolates on plant health. The results obtained showed that *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 had a positive impact on plant growth of tomato plants grown. Infection and mortality rates of 0% were observed for tomato plants grown on the three substrates inoculated with these two bacteria. No pathogenic fungi were isolated from the organs of tomato plants grown on the three substrates fermented with the bacteria. However, on untreated control plants, a variety of pathogenic fungi, namely *Fusarium* sp, *Phytophthora* sp, *Cladosporium* sp, *Rhizoctonia* sp, *Colletotrichum* sp, *Penicillium* sp, *Rhizopus* sp and *Trichoderma* sp were observed. However, the *Bacillus subtilis* KBM1 treatment stood out from *Bacillus venezensis* KBM1 with the best results for the growth parameters measured. In view of these results, these two bacteria could be used to produce an effective biofertilizer for tomato cultivation in Côte d'Ivoire.

Keywords: Tomato, fungal disease, bacterial biofertilizer, Côte d'Ivoire.

1. INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are annual plants belonging to the *Solanaceae* family [1]. It is the most widely consumed fruit after potatoes and the world's second most important food resource after cereals. This plant is adapted to a wide variety of growing conditions, in open fields or greenhouses [2]. With around 187 million tonnes produced worldwide in 2021, Africa accounted for 20 million tonnes of world production, and tomato production in Côte d'Ivoire was estimated at 40,306 tonnes, with a yield of 10293.4 kg per hectare [3]. Despite this production rate, yields are still low than the world's leading producer (China) and Africa's leading producer (Egypt), which are respectively 65 million tonnes and 6.7 million tonnes [3]. One of the causes of this low yield is the soil's lack of organic matter and phosphorus, which are the main constraints to intensified production [4]. In addition, the use of mineral or chemical fertilizer formulas that are not adapted to market gardening crops leads to unbalanced inputs and, in the long term, to the accumulation of certain heavy metals in the soil [5]. Indeed, numerous studies have shown the long-term negative effects of mineral fertilizers on soil fertility notably through their acidifying effect on the soil [6,7]. Growers also have to contend with various diseases that attack their crops. From many cash crops are ravaged by parasites, the most notorious of which are phytopathogenic fungi. The chemical products currently used against phytopathogenic fungi have their drawbacks. Most of them are toxic to users who come into contact with the preservative. This justifies the research currently being carried out in this field, with the aim of developing methods that are less harmful to the environment. As a result against these plant-pathogenic molds, through the use of microorganisms that produce substances with an antifungal effect. Biological is a very promising alternative, given the natural ubiquity of microbiological agents in ecosystems. The latter are characterized by their variety, ease of dissemination, specificity of action and persistence in the environment [8]. *Bacillus* species are considered to be important plant growth-promoting rhizobacteria (PGPR), producing a wide range of biologically active secondary metabolites potentially inhibit the growth of plant pathogens and rhizobacteria and deleterious rhizospheric microorganisms [9] can survive exposure, heat and desiccation, and their ability to be formulated into stable dry powders with a long shelf life [10]. Moreover, because this genus is already a common

inhabitant of the microflora of plant roots, biological control agents based on *Bacillus* spores have little or no effect on the composition of microbial communities in plant roots [11]. The bacterial species used in this study are strains designated as *Bacillus subtilis* KBM1 and *Bacillus velezensis* KBM1 isolated from the mango rhizosphere in Côte d'Ivoire. The aim of the study was to contribute to the improvement of tomato productivity in Côte d'Ivoire through the use of microbial organic fertilizers.

2. MATERIAL AND METHODS

2.1 Material

The material used in this study consisted, on the one hand, of tomato seed of variety UC 82 B developed by Green Seeds. and on the other, the biofertilizers *Bacillus subtilis* KBM1 and *Bacillus velezensis* KBM1 isolated from the rhizosphere of mango trees in Côte d'Ivoire and preserved in cryotubes in a freezer (-80°C). The substrates used in this study were soil and sawdust.

2.2 Methods

2.2.1 Preparation of the study plot

The soil in the plots was prepared before the various treatments were applied. This preparation consisted in cleaning a plot 5m long and 3m wide and weeding the surface. The sparse shrubs were then felled, and the trunks and branches were collected and disposed of in a landfill and discarded outside the plot boundaries using a wheelbarrow. Following this, tarpaulins were spread out along the plot, which was then delimited by Chinese bamboo stalks and covered with mosquito netting (Fig. 1).



Fig. 1. Study plot

2.2.2 Setting up the nursery

Sowing was carried out in a 77-cell plate, with three cells for each culture bag, in order to select the



most developed seedling at the end of the nursery for transplanting. One *Solanum lycopersicum* L tomato seed of the UC 82 B variety was sown in each alveolus at a depth of around one centimetre, followed by watering. Sowing was carried out in rows at distances of 5 to 10 cm. Watering was carried out twice a day, once in the morning and once in the evening. The honeycomb plates were then placed under a shade canopy and covered with straw for up to 21 days(Fig. 2).

Fig.2. Seeding

2.2.3 Biofertilizer production

One hundred (100) mL of yeast extract-peptone glucose (YPG) medium in two Erlenmeyer flasks were inoculated with a 48-hour-old culture. These pre-cultures were incubated at 30°C for 12 h with 105 rpm agitation. The pre-cultures were used to inoculate 2 L of YPG medium contained in two 1 L Erlenmeyer flasks. Two 1 L YPG media for each strain in the Erlenmeyer flasks were incubated with pre-cultures of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1. Media were stored at 30°C with 150 rpm agitation for 72 h, and biofertilizers were collected in sterile jars.

2.2.4 Cultivation design

The experimental design was based on the Fischer system[12]. A quantity of 100 mL of bacterial biofertilizer was added to 4.5 kg of each substrate. Thus, the treatments carried out in this study are represented by treatment T0 which was control 1 soil without biofertilizer, treatment T0' was control 2 containing soil mixed with sawdust without biofertilizer, treatment T0'' was control 3 and contained sawdust without biofertilizer, treatment T1 contained soil and 100 mL biofertilizer of *Bacillus subtilis* KBM1, treatment T2 contained soil mixed with sawdust and 100 mL of *Bacillus subtilis* KBM1 biofertilizer, and treatment T3 contained sawdust and 100 mL *Bacillus subtilis* KBM1 biofertilizer. The same treatments were carried out for the *Bacillus venezensis* KBM1 strain. Treatments were run in three replicates. Each replicate is represented by an agronomic bags. The agronomic bags set up for the trial are 50 cm apart. The experiment was carried out in 27 agronomic bags. These were arranged randomly in each block, under a greenhouse 5 m long and 3 m wide, i.e a surface area of 15m².

2.2.5 Plant transplanting

Once the system had been set up, the substrates were inoculated with the strains, after which the plants were transplanted into the various substrates then. After germination of the seeds, the were selected for cultivation. A total of 54 seedlings were transplanted, at a rate of 2 seedlings per agronomic bag. Transplanting took place on the 21st day after sowing. These seedlings, with 4 to 6 fully expanded leaves, were removed from the honeycomb plates and transplanted into the various agronomic bags (Fig.3).



Fig.3. Transplanting plants.

2.2.6 Measurement of agronomic parameters

Parameters measured included plant size, number of leaves, infection rate and mortality rate of tomato plants. Plant size, corresponding to stem length, was measured using a tape measure, while the number of leaves was counted manually every three days for 6 weeks. The infection rate expresses the percentage of diseased plants in relation to the total number of plants [13]. It was determined once a week for 6 weeks, using the formula :

$$\text{Infection rate(\%)} = \frac{\text{Total number diseased plants}}{\text{Total number of transplanted plants}} \times 100$$

- As for the mortality rate, it was calculated according to the following formula:

$$\text{Mortality rate(\%)} = \frac{\text{Total number of dead plants}}{\text{Total number of transplanted plants}} \times 100$$

2.2.7 Testing for tomato spoilage moulds.

The direct contact isolation technique on PDA agar as described by [14] was used to test tomato plants for spoilage moulds. Three altered leaves, stems and roots were randomly selected from each sample, then disinfected with 2% bleach for 2 min to eliminate exogenous microflora, and rinsed 2 times with sterile distilled water to remove bleach residues. Plant leaves, stems and roots are dried in sterile trays, then disinfected with absorbent cotton soaked in 70% ethanol prior to sampling. Three fragments of leaves, stems and roots were removed using a sterile scalpel. These fragments were placed separately on Petri dishes containing Potato Dextrose Agar (PDA). The plates were incubated at 28°C for 5 to 7 days. To obtain a pure strain, several subcultures were performed on PDA medium. Thus, a mold colony filament was taken using sterile forceps then placed at a single point in the center of a Petri dish containing the PDA medium in order to obtain typical mold development. Incubation is carried out at 28°C for 7 days. This method is repeated until pure colonies are obtained.

2.2.8 Identification of isolated fungi

Identification of molds was carried out on the basis of macroscopic and microscopic observations according to the method of [15]. Macroscopic Identification was carried out according to the method through an examination of culture on PDA. The cultural characteristics determined were the appearance of the colonies (fluffy, woolly, cottony, velvety, powdery or granular), the shape of the colonies (regular or irregular), the relief of the colonies (flat, convex, pleated, etc.), the color of the colonies (white, cream or colored, yellow, orange, brown, green, gray to black), colony size (small, extensive or invasive) and growth (rapid or slow). The isolated fungi were identified on the basis of their morphological characteristics according to the method described by [16]. To carry out the microscopic examination, a drop of methylene blue was placed on a clean slide, a small fragment of the isolate was placed on the drop using forceps and covered with a coverslip then observed under an electron microscope with an objective x 40 and the size of the conidiospores (short or long).

2.2.9 Statistical analysis

The results obtained were processed using Excel 2016 to calculate the averages. Secondly, R software version 3.2.2 was used to carry out analysis of variance (ANOVA) to highlight statistical differences between the averages obtained. In the event of a significant difference at the 5% threshold, the Tukey test was performed to determine the different homogeneity classes.

3. RESULTS

3.1 Effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on tomato plant mortality.

Fig.4 shows mortality rates of 0% for tomato plants grown on the three substrates inoculated with the two bacteria. However, those grown on substrates without bacteria showed mortality rates ranging from 6 ± 2.37 to 33 ± 3.15 % for soil and sawdust respectively.

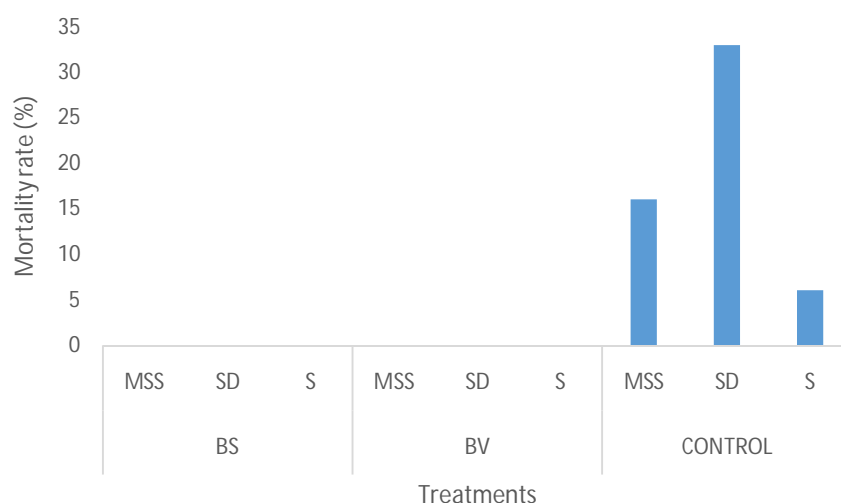


Fig. 4. Plant mortality rate (%) as a function of treatments

BS: Bacillus subtilis KBM1, BV: Bacillus venezensis KBM1, S: Soil, MSS: Mixed soil of sawdust and SD: Sawdust.

3.2 Effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on the infection rate of tomato plants.

Fig. 5 shows the development of disease symptoms on control plants compared with plants grown on substrates inoculated with the two bacteria, which showed no disease symptoms. Overall, it can be seen that all tomato plants grown on substrates without bacteria recorded infection rates of 26 ± 1.11 , 31 ± 2.17 and 42 ± 3.45 % for soil, sawdust and non-inoculated soil and sawdust respectively. However, tomato plants grown on substrates inoculated with *Bacillus* strains showed infection rates of 0%(Fig.6).

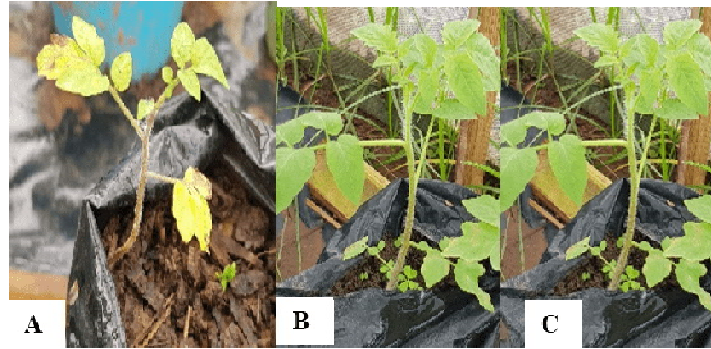


Fig. 5.Development of disease symptoms on tomato plants: A-Control, B-SBV and C-SBS. SBS: Soil with *Bacillus subtilis* KBM1, SBV: Soil with *Bacillus venezensis* KBM1

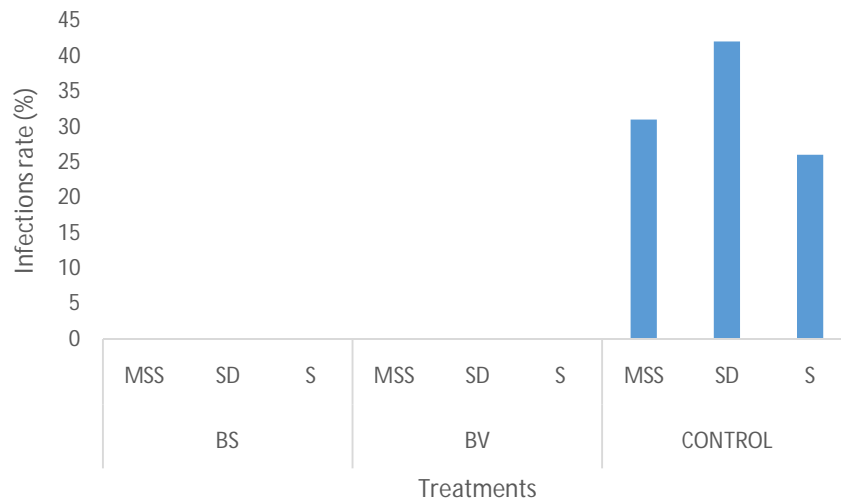


Fig. 6.Rate of infections of treated and untreated plants as a function of time (%). BS: *Bacillus subtilis* KBM1, BV: *Bacillus venezensis* KBM1, S: Soil, MSS: Mixed Soil with sawdust and SD: Sawdust.

3.3 Effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on tomato plant size.

Fig. 7 shows that inoculation with *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 strains had a highly significant impact ($p < 0.05$) on the evolution of tomato plant over the course of the experiment on the three substrates used. Control plants had low average heights, ranging from 9.93 ± 2.15 to 17.43 ± 2.24 m for sawdust and soil respectively. On the other hand, with the addition of the two

Bacillus strains, there was a definite increase in plant size, with the highest growth rates recorded at soil level, at 45.81 ± 1.18 cm for *Bacillus subtilis* strain KBM1 and 42.16 ± 2.22 cm for *Bacillus venezensis* strain KBM1. The growth rates obtained for *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 were $86.90 \pm 3.75\%$ and $85.76 \pm 2.43\%$ respectively, compared with $65.57 \pm 2.61\%$ for the soil control (Fig. 8).

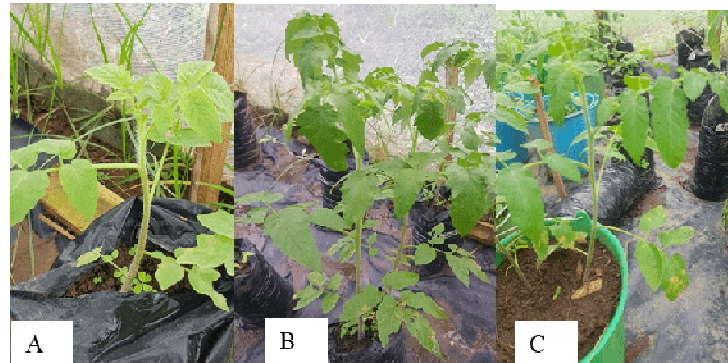


Fig.7. Size of tomato plants after 6 weeks: A- Control plant, B- Tomato plant with *Bacillus venezensis* KBM1 and C- Tomato plant with *Bacillus subtilis* KBM1.

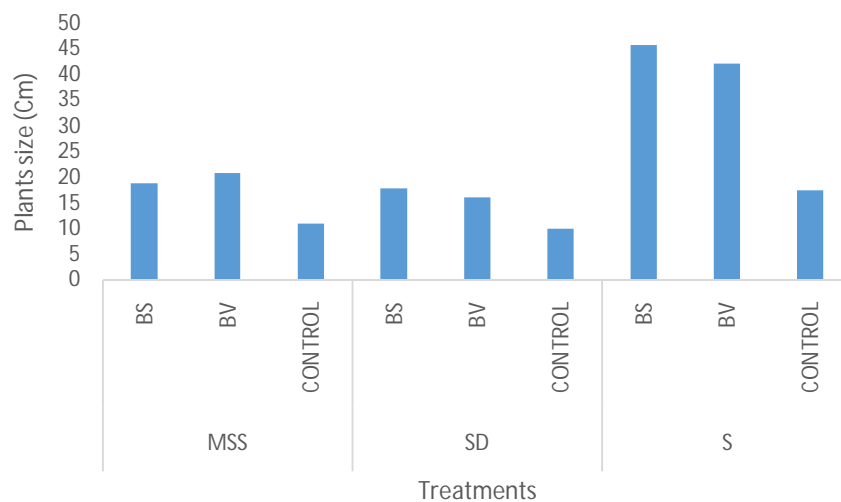


Fig.8. Effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on tomato plant size.

BS: *Bacillus subtilis* KBM1, BV: *Bacillus venezensis* KBM1, S: Soil,
MSS: Mixed soil of sawdust and SD: Sawdust

3.4 Effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on leaf numbers of tomato plants

The positive influence of *Bacillus* on the number of leaves on tomato plants is shown in fig. 9 and 10. Tomato plants grown on substrates supplemented with bacteria more leaves than control plants. The number of leaves significantly different from one substrate to another and according to bacterial strain ($p < 0,05$). Control plants showed leaf numbers ranging from around $21 \pm 2,5$, $23 \pm 3,5$ and $27 \pm 2,25$ respectively for plants grown on sawdust medium (SB), sawdust medium mixed soil (SSB) and soil (S). Tomato plants grown on substrates inoculated with inoculated substrates showed leaf numbers of $31,27 \pm 1,27$, $36,47 \pm 2,17$ and $70,07 \pm 1,48$ respectively for plants plants grown on sawdust (SB), sawdust mixed with soil (SSB) and soil (S).

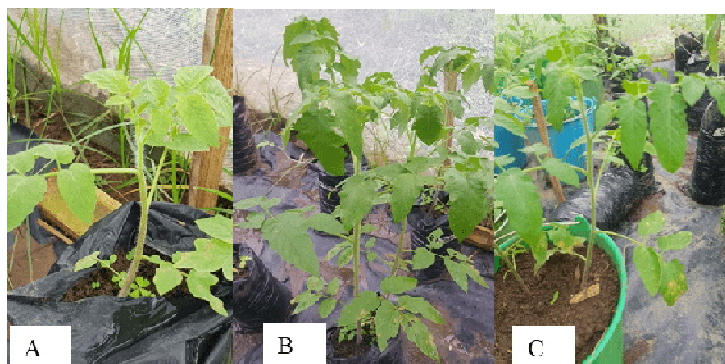


Fig. 9. Number of leaves of tomato plants after 6 weeks of cultivation: A- Control plant, B- Tomato plant with *Bacillus Venensis* KBM1 and C- Tomato plant with *Bacillus subtilis* KBM1.

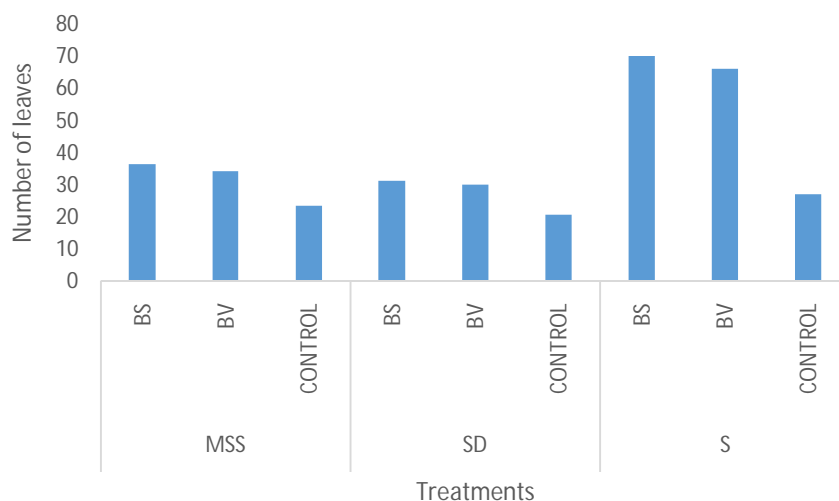


Fig.10. Effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 on the number of leaves of treated and untreated tomato.

BS: *Bacillus subtilis* KBM1, BV: *Bacillus venezensis* KBM1, S: Soil,
MSS: Mixed soil of sawdust and SD: Sawdust

3.5 Identification of weathering moulds on untreated tomato plants

The identification results showed the presence of pathogenic fungi on the roots, stems and leaves of tomato plants grown on substrates without fertilizing bacteria. However, no pathogenic fungi were isolated from samples of tomato plants grown on substrates inoculated with both strains of bacteria. 32 fungal isolates were assimilated to 8 genera, namely *Penicillium*, *Rhizopus*, *Fusarium*, *Trichoderma*, *Rhizoctonia*, *Cladosporium*, *Phytophthora* and *Colletotrichum*(Fig. 11, 12, 13, 14, 15, 16, 17, 18).

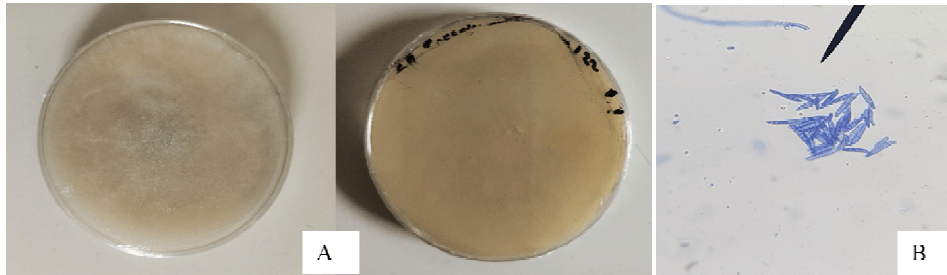


Fig.11. *Fusarium* sp: macroscopic appearance (A) and microscopic appearance (B) (GX10 ; methylene blue)

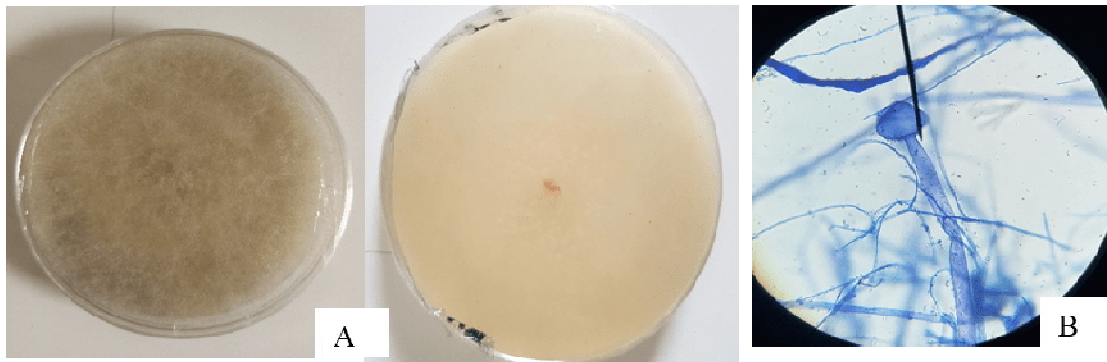


Fig. 12. *Rhizopus* sp: Macroscopic appearance (A) and microscopic appearance (B) (GX10 ; methylene blue)

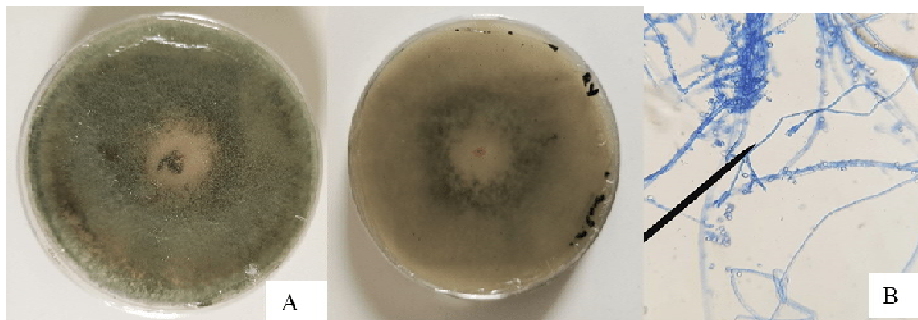


Fig. 13. *Penicillium* sp: Macroscopic appearance (A) and microscopic appearance (B)(GX10 ; methylene blue)

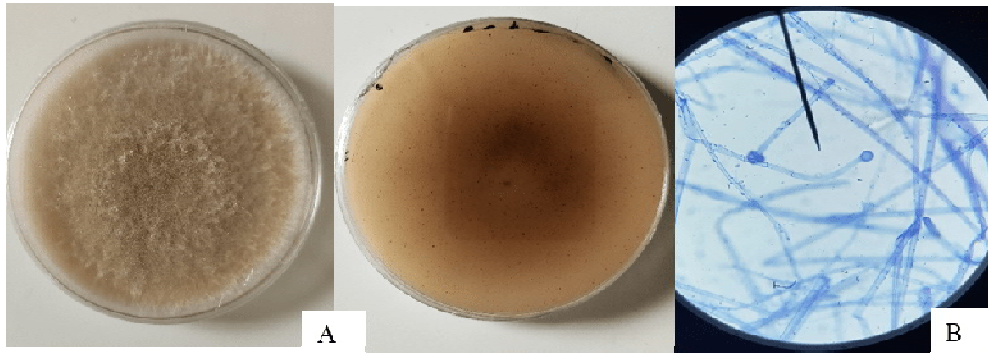
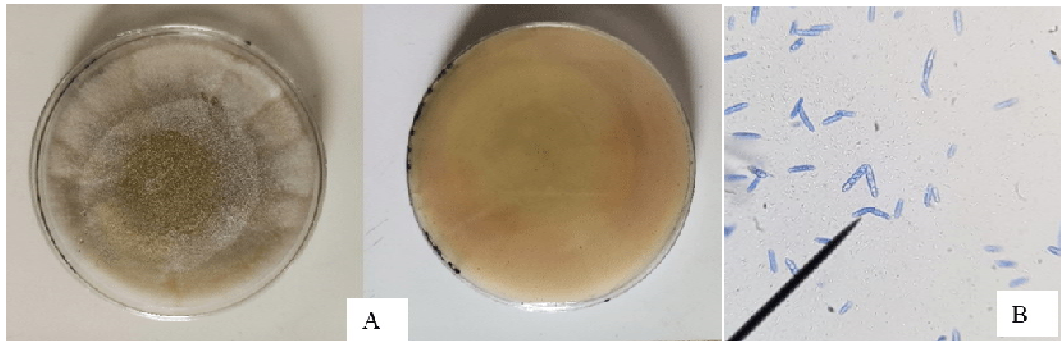
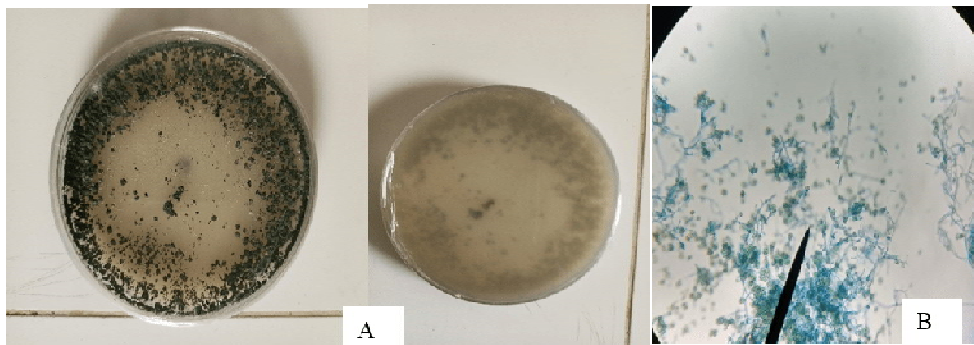


Fig. 14. *Phytophthora* sp: Macroscopic appearance (A) and microscopic appearance (B) (GX10 ;



methylene blue)

Fig. 15. *Cladosporium* sp: Macroscopic appearance (A) and microscopic appearance (B)



(GX10 ; methylene blue)

Fig. 16. *Trichoderma* sp: Macroscopic appearance (A) and microscopic appearance (B) (GX10 ; methylene blue)

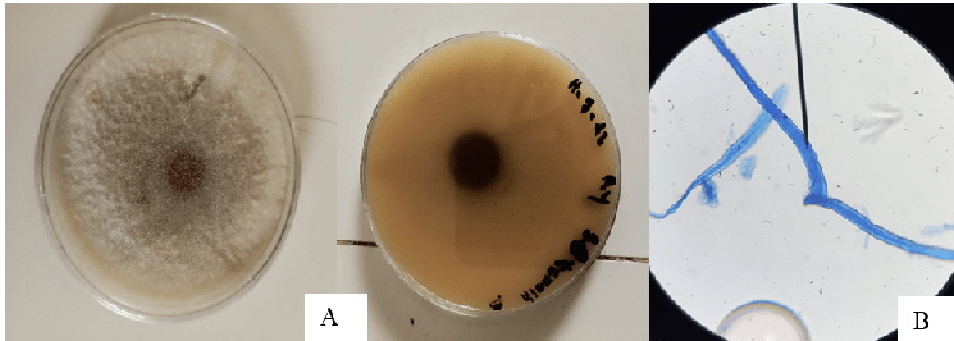


Fig. 17. *Rhizoctonia* sp: Macroscopic appearance (A) and microscopic appearance (B)(GX10 ; methylene blue)

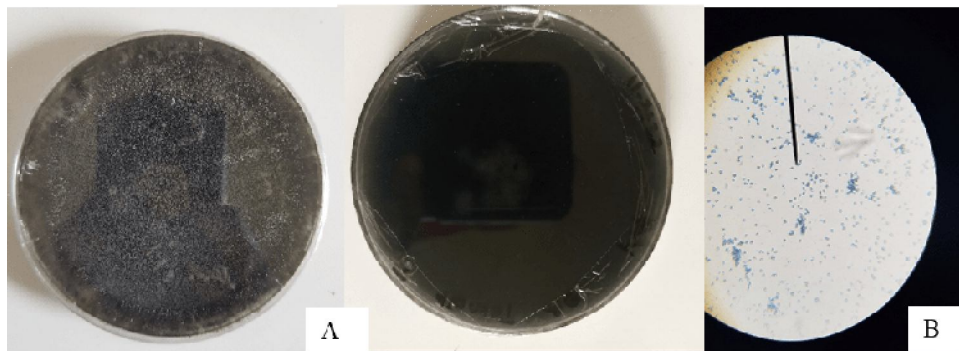


Fig. 18. *Colletotrichum* sp: Macroscopic appearance (A) and microscopic appearance (B)(GX10 ; methylene blue)

3.6 Frequency of isolation of fungi

Of the 32 mold isolates, *Fusarium* sp and *Rhizoctonia* sp dominated, with isolation frequencies of 25%. *Penicillium* sp, *Cladosporium* sp, *Phytophthora* sp and *Colletotrichum* sp showed isolation frequencies of 6.25%. The majority of fungi were found on all tomato plant organs except *Cladosporium* sp which was not isolated from stems and leaves. In addition, the frequency of isolation of fungi was on roots and stems, at 34.38% and 31.25% respectively (Table 1).

Table 1. Frequency of isolation of pathogenic fungi from tomato plants.

4. DISCUSSION

With the aim of improving tomato productivity, the stimulatory effect of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 in the growth of tomato plants and in the reduction of tomato diseases. Inoculation of substrates with the two bacterial strains to stimulate tomato plant growth by progressively increasing the size and number of leaves. This stimulation of tomato plant growth could be explained by the fact that the *Bacillus* strains facilitate better assimilation of plant nutrients. In the literature, the stimulating effect of *Bacillus* on plant growth is widely reported. The work of [17] has reported superior growth of tomato plants inoculated with *B. amyloliquefaciens* and *B. subtilis*. Bacteria of the *Bacillus*

FUNGAL ISOLATES	ISOLATING FREQUENCY BY ISOLATES (%)		ISOLATION FREQUENCY PER SAMPLE (%)		
	ISOLATES NUMBER	PERCENTAGE (%)	LEAF	STEM	ROOT
<i>Fusarium sp</i>	8	25%	30(3)	9,09(1)	36,36(4)
<i>Cladosporium sp</i>	2	6,25%	20(2)	0	0
<i>Phytophthora sp</i>	2	6,25%	10 (1)	0	9,09(1)
<i>Rhizoctonia sp</i>	8	25%	40 (4)	9,09(1)	27,27%(3)
<i>Trichoderma sp</i>	3	9,37%	0	9,09(1)	18,18(2)
<i>Penicillium sp</i>	2	6,25%	0	9,09(1)	9,09(1)
<i>Rhizopus sp</i>	5	15,63%	0	45,45(5)	0
<i>Colletotrichum sp</i>	2	6,25%	0	18,18(2)	0
TOTAL	32	100,00%	31,25%(10)	34,38%(11)	34,38%(11)

genus are recognized for their direct mode of action (phytostimulatory effect), which stimulates plant growth by increasing aerial and root mass, root elongation and accelerated seedling emergence, have undoubtedly made available to plants the nutrients that were available in the soil but which they were unable to use. Nitrogen-fixing rhizobacteria such as the *Bacillus* genus are important for good soil fertilization and a sustainable agricultural system. These results are supported by several authors, including [18], who showed that nitrogen levels have a significant effect on potato leaf yield. Some bacteria are capable of producing enzymes such as phosphatase, or organic acids capable of converting phosphorus into forms usable by plants: $H_2PO_4^-$ and HPO_4^{2-} . Phosphorus solubilization is the most common mode of action of PGPR rhizobacteria capable of improving nutrient availability [19]. As such, certain *Bacillus* have been studied for this capacity on various crops by [20]. Solubilizing rhizospheric bacteria phosphate (PSB) could therefore constitute a promising source as an agent of biofertilizers in agriculture. The results of this study show that the strains of *Bacillus subtilis* KBM1 (BS) and *Bacillus venezensis* KBM1 (BV) influence the attack of tomato plants by fungi with reduction and infection rates of 0%. The addition of strains of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 significantly reduced the action of several tomato pathogens on treated plants originating from substrates inoculated with the bacteria. It has been shown by several authors that organic soil fertilizers can suppress diseases caused by soil-borne pathogens such as *Rhizoctonia* [21]. This suppression has often been attributed to a compost microflora such as *Bacillus* and fungi, antagonistic to soil-borne phytopathogens [22]. Numerous works present the diversity of microbial agents involved in biological control which can suppress a wide spectrum of bacterial, fungal and parasitic diseases where the *Bacillus* genus is cited [23]. The beneficial effect of *Bacillus* on the reduction of attacks is associated with an increase in the resistance of plants to colonization by the pathogen due essentially to the formation of physical barriers at the penetration sites of the fungus [24]. These results are corroborated by the study of [25], which showed an increase in the survival of tomato seedlings inoculated with *B. subtilis* RB14-C. The application of *Bacillus* in the soil not only protects plants against soil-borne diseases, but can also strengthen the overall health status of plants by inducing systemic resistance (ISR) in the plant [26]. During the phenomenon called "induced systemic resistance" (ISR), non-pathogenic rhizobacteria, notably certain species of *Bacillus*, can confer on the plant a certain degree of protection against subsequent attacks by a phytopathogen via the stimulation of systemic defense mechanisms. This immunity is initiated following the perception by the plant of so-called elicitor molecules produced by the beneficiary microorganism. These results corroborate with

those of [27] who showed in their work that rhizobacteria interact with the roots of the host and produce elicitors which are perceived by the plant. After the recognition of the determinants, a signal is conveyed throughout the plant in order to alert it and finally, during a possible attack by a phytopathogenic agent, the plant will be able to respond more effectively to the attack, thus giving it resistance. The second part of this study aimed to test the effectiveness of *Bacillus subtilis* KBM1 and *Bacillus Venensis* KBM1 on the reduction of tomato diseases in Côte d'Ivoire. The results obtained following the phenotypic identification of fungal strains isolated from the leaves, stem and root of tomato plants from infected controls made it possible to show that a diversity of molds are responsible for the deterioration of the pre-harvest in Côte d'Ivoire. Eight (8) types of mold have been identified, namely *Fusarium* sp, *Rhizoctonia* sp, *Cladosporium* sp, *Colletotrichum* sp, *Phytophthora* sp, *Trichoderma* sp, *Rizopus* sp, and *Penicillium* sp. Among these isolated strains, many are recognized as pathogens of tomatoes in pre- or post-harvest. *Colletotrichum* sp by example that was isolated from the tomato stem in our study is similar to one of these species (*Colletotrichum coccodes*) which is responsible for anthracnose on tomato fruit. "anthracnose" or root rot or anthrax is a disease induced by *Colletotrichum coccodes* which causes damage to roots, leaves, stems and fruits[28]. Recognized as a pathogen specific to tomato, *Cladosporium fluvum* or *Passalora fulva* is responsible for the disease called olive mold or cladosporiosis in tomatoes. The causative agent of olive mold was *Passalora fulva*[29], it seems to present great affinities for the tomato, in particular for its leaflets but remains harmful for this one. A recently defined disease in tomatoes, downy mildew is one of the main aerial diseases of tomato cultivation, particularly when conditions are cool and damp. The phytopathogenic agent of this disease is *Phytophthora infestans*. [30]to show in his studies that during the progression of the disease due to *Phytophthora* infest, the foliage gradually turns yellow then brown, curls then shrivels before die. As for *Fusarium* sp, it is responsible for root and crown rot and creates *Fusarium* wilt in tomatoes. *Fusarium* is responsible for vascular wilting by their invasion of the xylem vessels[31]. There tomato can be attacked by two different *Fusarium* diseases, *Fusarium* wilt caused by *Fusarium oxysporum lycopersici* and root and crown rot caused by *Fusarium oxysporum radialis-lycopersici*. *Rhizoctonia* sp is also recognized in the crown rot and root rot of tomato. The genera of *Penicillium* sp, *Trichoderma* sp and *Rhizopus* sp are recognized as saprophytic tomato molds, responsible for fruit rot [32]. Inoculation of *Bacillus* strains into plants has significantly reduced tomato diseases. The same studies were carried out with [17] who measured the effectiveness of four *Bacillus* (*B. megaterium* MB3, *B. subtilis* strains MB99 and *B. subtilis* MB14 and *B. amyloliquefaciens* MB101) against *Rhizoctonia solani* on tomato. The authors measured the production of systemic defense enzymes, which increases following the application of certain *Bacillus* treatments: β -1,3-glucanases, chitinases (PR-3), phenylalanine ammonia lyase (PAL), and proteases. The same authors also revealed that *Bacillus subtilis* MB14 provided 60% inhibition of the disease as well as greater growth in the presence of the pathogen. In our study, the results showed that the strains of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 therefore reduced diseases in tomato by a 0% of infection and mortality in treated plants.

5. CONCLUSION

The aim of this study was to contribute to improving tomato productivity in Côte d'Ivoire through the use of organic fertilizer of microbial origin. The results obtained showed that the strains of *Bacillus subtilis* KBM1 and *Bacillus venezensis* KBM1 stimulated the growth of tomato plants through an increase importance of the growth parameters studied. These two bacteria also showed their ability to protect tomato plants through 0% infection and mortality rates for plants cultivated on the three substrates inoculated with the two strains of *Bacillus*. However, the *Bacillus subtilis* KBM1 strain was more effective than *Bacillus venezensis* KBM1. In view of these results, these two strains prove useful for making of a biofertilizer to improve tomato productivity in Côte d'Ivoire.

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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