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Influence of Microorganisms Effective against Basal Rot and on Agronomic Parameters of Onion [*Allium cepa* L. (Amaryllidaceae)]

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Abstract

Onions are a horticultural crop of great economic, dietary and medicinal importance, and are highly prized by the Ivorian population. However, production remains low, due to a number of constraints, including parasitic attacks. The most frequent is fusariosis caused by *Fusarium* sp., a pathogen that causes enormous damage to onion crops. Faced with these attacks, chemical control appears to be ineffective, with consequences for human health and the environment. This is why the search for effective alternative methods that respect the environment and human health is so necessary. It is in this context that this study was carried out, with the general aim of controlling fusarium wilt in onion crops, with a view to improving onion production in Ivory Coast through the use of effective microorganisms. The experimental set-up used for this purpose was a fisher block with complete randomization, comprising three replicates. A fungal spore concentration of 106 spore/mL of *Fusarium* sp., three doses (1%; 2.5% and 5% v/v) of EM and one dose of a chemical fungicide (30 mL/16L) were tested on young onion plants. Each block consisted of nine sub-plots with nine treatments. Health parameters (incidence and severity) and agronomic parameters (growth and yield) were assessed. Microbiological analysis of the EM revealed the presence of nine morphotypes of *Trichoderma* sp., *Aspergillus clavatus*, *Aspergillus flavus*, *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., lactic acid bacteria of the *Bacillus* family and the yeast *Saccharomyces cerevisiae*. Field experimentation showed that the 5% EM microbial solution reduced the incidence and severity of fusariosis compared with the chemical fungicide, and proved to be the best. This dose reduced yield losses by

7.14%, while improving onion growth and yield by over 5%. The results demonstrated the ability of the EM solution to effectively control the causal agent of basal rot in onion crops.

Keywords

Basal Rot, Effective Microorganisms (EM), *Fusarium* sp., Onion

1. Introduction

The onion (*Allium cepa* L., 1753) is one of the first vegetables domesticated by man. It is a horticultural crop [1] whose production is increasingly growing worldwide. In 2020, it was estimated at 105 million tonnes, with China, India and the United States as the world's main producers [2].

In West Africa, production was estimated at 3.5 million tonnes in 2020, with Nigeria, Niger and Senegal as the main producers [2].

Onion bulbs are used for human consumption in various forms (whole, peeled, crushed or cut) throughout the world. In addition to their nutritional value, they also have medicinal importance [3].

In 2021, Ivory Coast will import 189,012 tonnes of onions from the Netherlands.

Netherlands (Dutch Onion Association)

Onions have become Ivory Coast's most imported food product in terms of quantity, alongside rice and wheat. This makes Ivory Coast one of the smallest onion-producing countries in the sub-region, despite its considerable production potential in both savannah and forest areas. As a result, 95% of onions consumed come from outside the country. The slow development of the onion industry in Ivory Coast is due to the reluctance of certain populations to practice it [4]. To reduce onion dependence on the outside world, major production basins have been set up in most towns (Tingrela, Odienné, Korhogo, Bouna, Boundiali, Niakara, Dabakala, Katiola…) in Ivory Coast. But these don't seem to be growing as fast as consumption. This is partly due to the fact that onion production is threatened by fungi, bacteria, viruses, nematodes and pests that cause diseases from the nursery to storage causing significant losses [4].

Among these diseases is basal rot, caused by the soil-borne fungus Fusarium sp. which affects Allium species worldwide [5] [6]. The latter is common and proves very devastating as it can cause up to 70% of damage in nurseries, 50% of bulb losses in the field and 30% - 40% in storage [7] [8].

Measures to control these pathogens include seed and soil disinfection, crop rotation, the use of resistant varieties or synthetic chemical fungicides [9].

However, chemical fungicides are still the most widely used. This has consequences for the health of farmers, consumers and the environment [10]. The

search for effective alternative methods that respect the environment and human health is becoming increasingly necessary. This is where the EM (Efficient Microorganisms) technique comes into play, as it is considered a practice that supports natural agriculture thanks to beneficial microorganisms. These include photosynthetic bacteria, lactobacilli, yeasts, actinomycetes and aerobic and anaerobic non-pathogenic fungi [11]. These microorganisms improve soil structure while promoting improved growth, crop yield and soil disease control [12] [13]. It was in view of the advantages offered by this technique that the present study was initiated.

The general objective of this study is to improve onion production in Ivory Coast through the use of effective microorganisms.

More specifically, the aim was to identify the various microbial components of the microbial solution, and assess its effectiveness in combating basal rot and its impact on onion production.

2. Materials and Methods

2.1. Experimental Site

The study was conducted on the experimental plot of Nangui Abrogoua University (UNA). Geographically the experimental field was located at: 5˚17'N and 5˚31"N latitude and 3˚45'W and 4˚31'W longitude. The site was characterized by moderate and high temperatures (24.51˚C to 27.67˚C) and relative humidity of 80% during the experiment.

2.2. Materials

❖ **Vegetal material**

Damani violet of the Galmi type acquired at semi-variety was used as plant material for this study. This variety reaches maturity between 100 and 110 days.

❖ **Fongic material**

On the other hand, pathogenic *Fusarium* sp. fungal material was isolated from onion bulbs collected at the Adjame wholesale market showing white mycelium and rotting symptoms.

2.3. Methods

2.3.1. Preparation of the Microbial Solution (EM)

The preparation of the microbial solution (EM) was essentially carried out in three steps following the method described by [14].

The first step consisted of precooking the six starch sources separately and spreading them in perforated trays. The trays were then covered with fine cloth and placed individually in pits 20 to 30 cm deep, protected from disturbance and covered with Chinese bamboo leaf, then black bag and finally soil, for five days. After this incubation time, the contents of each tray were collected after eliminating areas with a grey, brown or black coloration characteristic of the presence of harmful microorganisms, while retaining those with a pink, green, white,

blue, yellow or orange coloration for EM preparation.

The starch sources colonized by the microorganisms (15 kg) were mixed with 15 kg of brown sugar and stored in a hermetically sealed 70 L plastic barrel under a shed for 15 days, after which the mixture became compact.

Next, 2 kg of unbleached local rice were washed with rainwater. After five days in a closed container, one liter of this washing water was added to 10 L of fresh cow's milk in a plastic barrel. The container was hermetically sealed and stored for seven days away from heat and light for fermentation, after which only the liquid part was extracted.

Finally, the dough and liquid obtained in steps 1 and 2 respectively were mixed, to which 100 g of baker's yeast and 20 liters of rainwater (chlorine-free) were added. Seven days later, the mixture was filtered and stored in a 200-liter plastic container, out of direct sunlight.

2.3.2. Isolation and Identifying of Microorganisms from the EM Solution

The isolation and enumeration of fungi, bacteria and yeasts from the EM solution was carried out using the suspension dilution method [15].

- **Suspension-dilution preparation and isolation**

From 10 mL of EM extract, vortexed for 20 min and adjusted to 100 mL in a sterile Beaker, the stock solution (1/10, *i.e.* 10^{-1}) is obtained. Using a sterile graduated pipette, 1 mL of the stock suspension is removed and transferred to a sterile test tube containing 9 mL of sterile distilled water, numbered according to the 10^{-2} dilution.

All further dilutions up to 10^{-8} are obtained by the same procedure. One µL of each dilution was taken and spread on the surface of the agar culture medium (PDA) contained in the Petri dish and incubated for 48 h at 28˚C. Only Petri dishes with a dilution of 10^{-8} were used.

As for *Fusarium*, isolation was carried out on PDA culture medium according to the method used by [16]. Explants were collected in the laboratory from the growth front of infected onion bulb parts and disinfected for three minutes with a 2% sodium hypochlorite solution. The fragments obtained were then rinsed three times in succession with sterile distilled water for 5 min, then placed on sterile blotting paper to remove excess water. These were then inoculated onto PDA culture medium at a rate of three fragments per dish. They were sealed with stretch tape and incubated for 48 to 72 hours at room temperature. Strains were purified after several successive subcultures on PDA medium.

- **Methods for identifying microorganisms in Ems**

Fungi were identified 7 days after seeding on PDA medium. Macroscopic and microscopic characteristics were recorded using the identification keys of [17]-[19]. As for microscopic characteristics, Gram staining was used to classify bacteria according to their Gram, morphology and mode of association. Gram-positive and catalase-negative bacteria are presumed to be lactic acid bac-

teria [20]. Bacterial cells were observed at ×1000 magnification using a Motic light microscope [21].

- **Yeasts**

Agar medium (PDA) at pH 3.5 (adjusted with 10% tartaric acid) was used for its ability to support colony formation by *Saccharomyces* cells that were contained in the EM solution.

The sample of EM solution was serially diluted in 0.1 M phosphate buffer (pH 7.0) before being spread in recovery agar using the casting plate technique. Colonies were counted after five days' incubation at 29˚C [22].

2.3.3. Setting up the Experiment

- **Nursery sowing**

Nursery sowing was carried out under glass in three honeycombed trays, each with seventy-two holes filled with potting soil, purchased from SEMIVOIRE. Three seeds were then placed in each hole and covered with potting soil. Each plate received 600 mL of tap water per day until emergence, after which the seedlings were watered as required until transplanting. The insecticide Cypercal was applied once at a rate of 3.2 mL/600mL water for the three plates when caterpillar damage was observed three weeks after sowing.

- **Experimental set-up**

The experimental set-up used was a Fisher block with complete randomization, comprising three blocks, *i.e.* three replicates. Each block was made up of nine sub-plots with nine treatments. The treatments were as follows: T0 (no inoculation), T01 (2.5% of EM + *Fusarium* sp.), T1 (1% of EM), T2 (5% of EM + *Fusarium* sp.), T3 (2.5% of EM), T4 (*Fusarium* sp.), T5 (1% of EM + *Fusarium* sp.), T6 (5% of EM) and T7 (*Fusarium* sp. + chemical fungicide) with a concentration of *Fusarium* sp. (F: 106 spore/mL).

Each subplot contained eleven (11) plants, for a total of 297 plants. Plants were spaced 0.33 m apart in width and 0.5 m apart in length. Subplots were spaced 0.5 m apart and 1 m between blocks.

- **Transplanting, inoculation and care of seedlings**

The trial was conducted in a 7 × 6 × 3 (length × width × height) greenhouse, and nursery seedlings were exposed to the open air in a 14 m × 8 m plot. Forty-five days later, they were transplanted into two-liter plastic pots containing a substrate (sterilized in an oven for 30 minutes) made up of compost (pig droppings, legumes, poultry droppings, grass clippings) and UNA forest soil at a ratio of 1:2 (v/v). Plants received tap water as needed. Yara Mila fertilizer was applied at a dose of 3 g/pot two weeks after transplanting. Insecticide was applied at a rate of 40 mL/16L.

Inoculations were carried out in the greenhouse one month after transplanting, according to the treatments, while avoiding watering the plants on the day of inoculation. Only one inoculation per plant was made with the 106/mL spore suspension of the pathogen *Fusarium* sp. and four inoculations with EM. A single inoculation was made 24 h after inoculation of the plants

with the pathogen, and then every week for three weeks. During the trial, the T0 control was treated with tap water only. Banko-plus was applied four times (30 mL/16L).

2.3.4. Evaluated Parameters

❖ **Health parameters**

- Incidence was calculated as follows [23]:

$$\text{Incidence rate}(\%) = \frac{\sum n}{N} \times 100 \quad (1)$$

where: $\sum n$: sum of diseased plants; N: total number of plants observed.

- Disease severity was assessed using the symptom rating scale of Vakalounakis and Fragkiadakis by [24] on cucumber, adapted by Dabiré in 2016 [16] on onion fusariosis using the formula of [23].

$$\text{Severity}(\%) = \frac{\sum \text{note}}{Nt \times 3} \times 100 \quad (2)$$

with: $\sum \text{note}$: sum of different notes for infected plants; Nt: total number of infected plants.

❖ **Agronomic parameters**

The agronomic parameters assessed were growth (plant height, number of leaves, plant collar diameter) and yield of fresh onion bulbs calculated according to the following formula:

$$\text{Rendement}\left(\text{kg} \cdot \text{ha}^{-1}\right) = \frac{\text{Rendement parcellaire}}{\text{Superficie parcellaire}} \times 10000 \quad (3)$$

❖ **Statistical analysis**

All data collected was entered into an Excel spreadsheet. Graphs were designed for illustrative purposes. For comparative analysis, data were tested for normality and homogeneity using the Shapiro-Wilk and Leven tests. If these conditions were not met, the Kruskal-Wallis test was performed. Otherwise, the Newman-Keuls multiple comparison test was used to compare means when the analysis of variance (ANOVA 1) revealed significant differences. XLSTAT version 2016 software was used for these analyses, and statistical tests were performed at the 5% probability threshold.

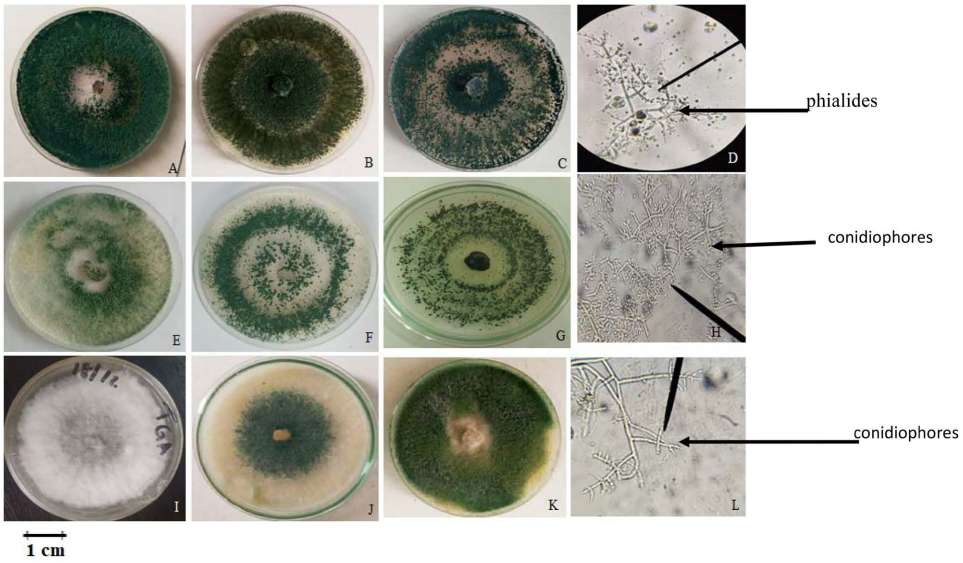
3. Results

3.1. Microbiological Analysis of the EM Solution

3.1.1. Fungi

Four fungal genera were identified from the EM solution using the key [18]. These were the genera *Trichoderma*, *Aspergillus*, *Penicillium* and *Rhizopus*. The genera *Trichoderma* and *Aspergillus* presented several morphotypes with different cultural aspects (**Figures 1-4**).

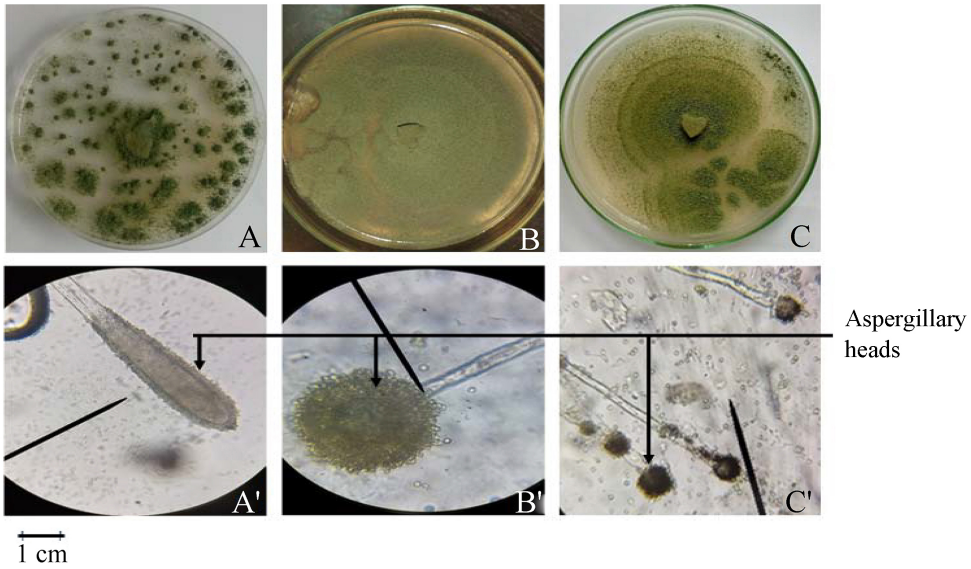
- ***Trichoderma* sp.**



A, B, C, E, F, G, I, J, K: faces of different Petri dishes D, H, L: microscopic observations (×400): conidiophores, phialides.

Figure 1. Macroscopic and microscopic views of *Trichoderma* sp.

- ***Aspergillus* sp.**

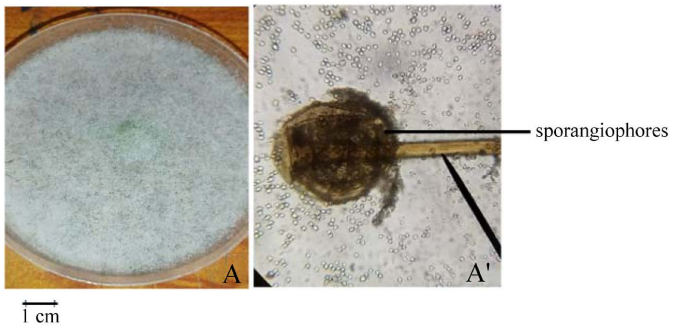


A: *Aspergillus clavatus*; B: *Aspergillus flavus* and C: *Aspergillus* sp.; A', B' and C': microscopic observations (×400): phialides and basipetal conidia.

Figure 2. Macroscopic and microscopic views of *Aspergillus* sp.

- ***Rhizopus* sp.**

Rhyzopus sp. colonies were white filaments with black spores at the tip. Microscopically, they showed an unpartitioned, unbranched mycelium with spores and branched sporangiophores at the end of the mycelium. Spores are found on the aerial part of the mycelium and surrounded by vesicles (**Figure 3**).

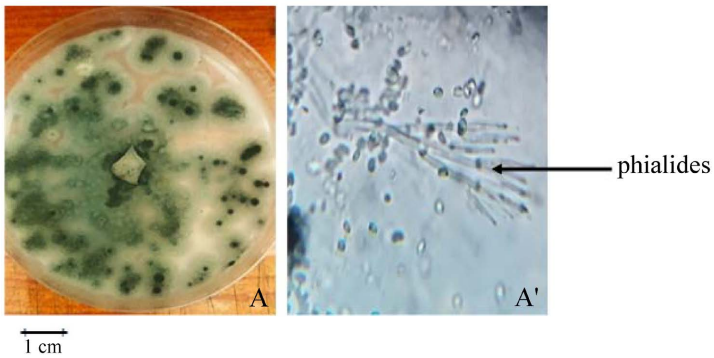


A: face of Petri dish; A': microscopic observation (×400): sporangiophores.

Figure 3. Macroscopic and microscopic views of *Rhizopus* sp.

- ***Penicillium* sp.**

The colony was flat and flaky, green-grey in color. Microscopically, the thallus, formed of hyaline septate mycelial filaments, bears conidiophores. Phialides are arranged in whorls at the tips of the conidiophores, and the latter are grouped in bundles (**Figure 4**).



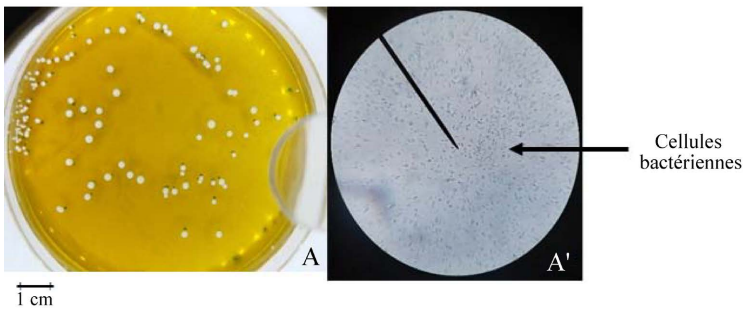
A: face of Petri dish; A': microscopic observation (×400): mycelium and phialides.

Figure 4. Macroscopic and microscopic views of *Penicillum* sp.

3.1.2. Lactic Acid Bacteria

Colonies grown on MRS medium were small, white and round in shape. They were Gram-positive and did not produce hydrogen peroxide (H_2O_2) (**Figure 5**).

- **Bacterial cells**

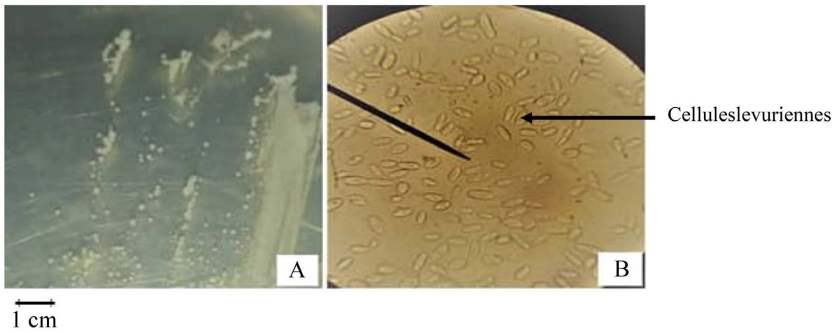


A: colonies of lactic acid bacteria; A': bacteria seen under the light microscope (×1000).

Figure 5. Macroscopic and microscopic views of lactic acid bacteria.

- **Yeasts**

Colonies were white, creamy, smooth and shiny. Microscopically, the cells were ovoid (Figure 6).



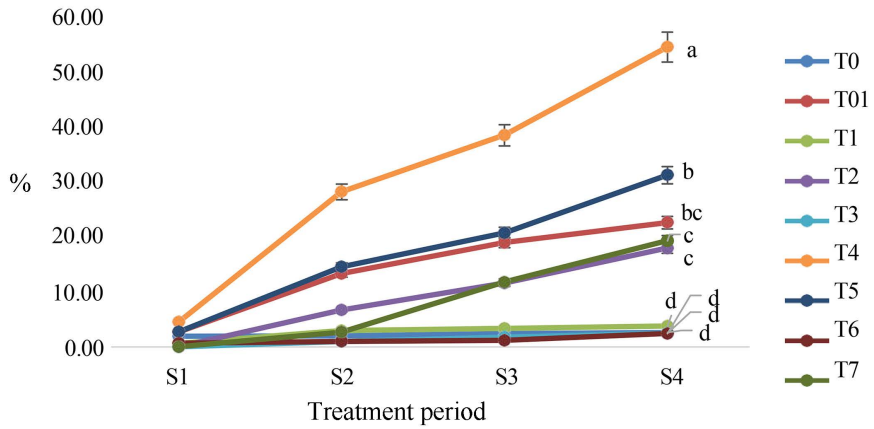
A: colonies viewed with an ocular magnifying glass; B: cells viewed with a light microscope (×400).

Figure 6. Macroscopic and microscopic views of *Saccharomyces cerevisia*.

3.2. Effect of Effective Microorganisms (EM) on Incidence and Severity

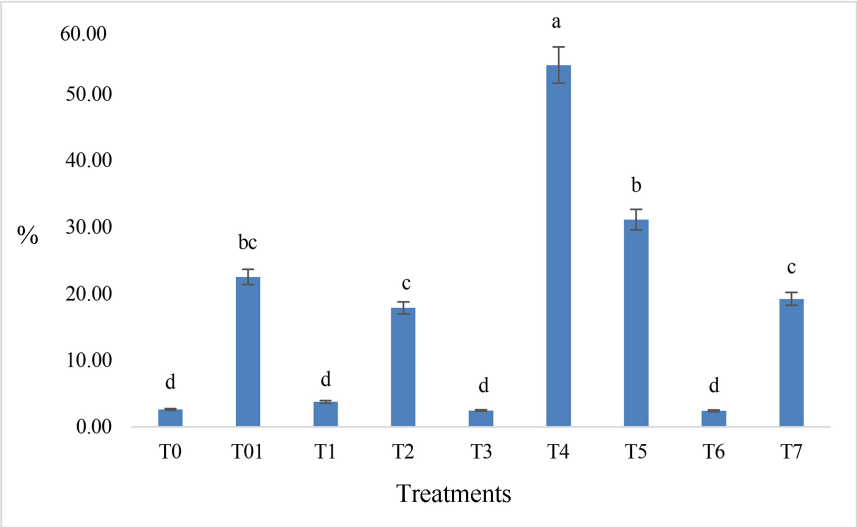
3.2.1. Fusarium Incidence on Onion Plants

Incidence rates varied according to the treatment applied (Figures 7-8). Plants inoculated with *Fusarium* spores and treated had a lower incidence rate than those inoculated with Fusarium spores only. However, treatment T2 inoculated and treated with 5% EM recorded an incidence rate of 17.90%, statistically identical to that of T7 (19.25%) inoculated and treated with chemical fungicide. The highest incidence rates were recorded in treatments T4 (54.42%), T5 (31.18%) and T01 (22.56%). Low incidence rates were recorded in treatments T6, T3, T1 and T0.



Means of disease incidence followed by the same letter show no significant difference at the 5% level (Newman-Keuls test). T0: control; T01: *Fusarium* sp. + 2.5% EM; T1: 1% EM; T2: *Fusarium* sp. + 5% EM; T3: 2.5% EM; T4: *Fusarium* sp.; T5: *Fusarium* sp. + 1% EM; T6: 5% EM; T7: *Fusarium* sp. + chemical fungicide. Mean ± standard deviation.

Figure 7. Changes in the incidence of Fusarium head blight as a function of treatments.

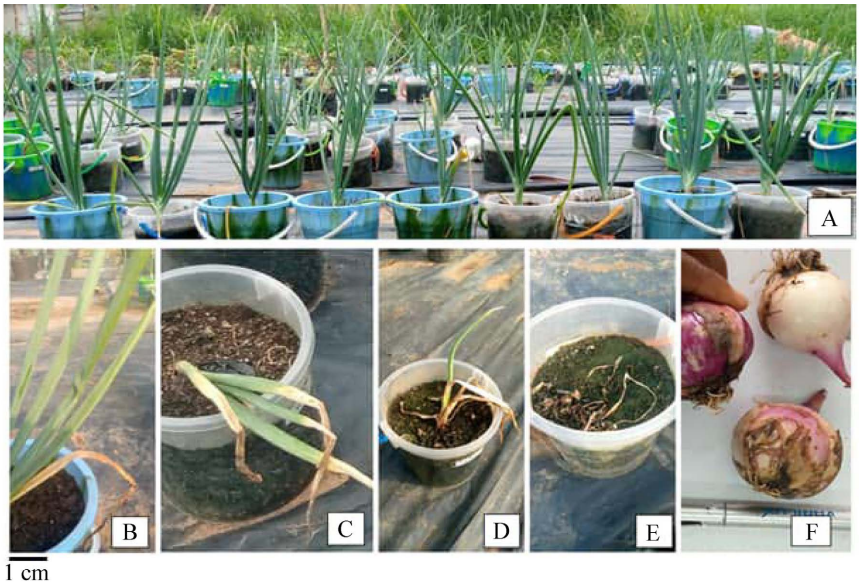


Means of disease incidence followed by the same letter show no significant difference at the 5% level (Newman-Keuls test). T0: control; T01: *Fusarium* sp. + 2.5% EM; T1: 1% EM; T2: *Fusarium* sp. + 5% EM; T3: 2.5% EM; T4: *Fusarium* sp.; T5: *Fusarium* sp. + 1% EM; T6: 5% EM; T7: *Fusarium* sp. + chemical fungicide. Mean ± standard error.

Figure 8. Incidence of fusariosis on onion plants according to treatments.

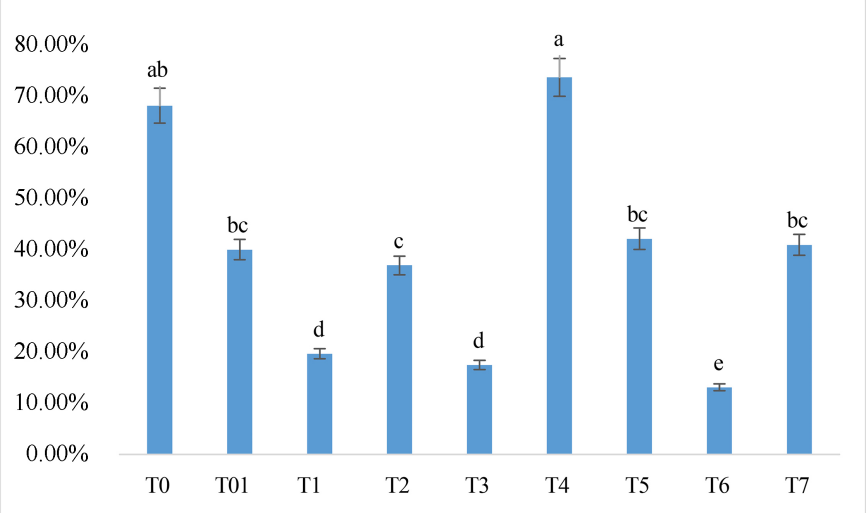
3.2.2. Fusarium Head Blight Severity

Figure 9 shows the different health conditions of the plants while **Figure 10** shows the severity of the disease depending on the treatments. Treatments showed different levels of disease severity, ranging from 16.67 to 70.31%. Treatment T2 showed the lowest severity rate (35.23%) compared with control T4 (70.31%), *i.e.* a reduction of 35.08%. On the other hand, disease severity was higher in treatments receiving no dose of EM or chemical fungicide. In these



A: healthy plants; B: beginning of yellowing of leaves; C: drying out of leaves; D: towards plant senescence; E: dead plant; F: rotting of harvested bulbs.

Figure 9. Typical symptoms of fusarium base rot of *Allium* sp.



Disease severity means followed by the same letter show no significant difference at the 5% level (Newman-Keuls test). T0: control; T01: *Fusarium* sp. + 2.5% EM; T1: 1% EM; T2: *Fusarium* sp. + 5% EM; T3: 2.5% EM; T4: *Fusarium* sp.; T5: *Fusarium* sp. + 1% EM; T6: 5% EM; T7: *Fusarium* sp. + chemical fungicide. Mean ± standard error.

Figure 10. Effect of fusarium severity on onion plants.

treatments, disease severity was between 65 and 70%. As for treatments that received only the various doses of EM, their severity ranged from 12% to 18%.

3.3. Effect of Effective Microorganisms (EM) on Agronomic Parameters

3.3.1. Growth Parameters

The effect of treatments on the three growth parameters is listed in **Table 1**.

Table 1. Growth parameters according to treatments.

Treatments	NF	H	D
T0	6.15 ± 2.3 b	35.91 ± 8.7 b	7.02 ± 2.5 b
T01	5.94 ± 2.6 b	32.43 ± 9.1 d	6.86 ± 3.2 b
T1	6.17 ± 2.5 b	36.27 ± 8.6 ab	7.34 ± 3.0 a
T2	5.97 ± 2.3 b	34.99 ± 9.8 c	6.9 ± 2.9 b
T3	7.33 ± 2.4 ab	36.77 ± 5.2 a	7.57 ± 3.4 a
T4	4.3 ± 2.3 c	27.72 ± 9.0 e	5.24 ± 2.4 c
T5	5.69 ± 3.0 b	31.65 ± 8.9 f	6.61 ± 3.9 bc
T6	8.57 ± 2.1 a	36.84 ± 6.7 a	7.58 ± 2.5 a
T7	6.0 ± 2.3 b	35.69 ± 9.5 b	6.92 ± 3.5 b
Pr	0.039	0.024	0.041

In the same column, the averages for number of leaves, plant height and plant diameter followed by the same letter show no significant difference at the 5% threshold (Newman-Keuls test). NF: number of leaves; H: leaf length; D: diameter at collar; T0: control; T01: *Fusarium* sp. + 2.5% EM; T1: 1% EM; T2: *Fusarium* sp. + 5% EM; T3: 2.5% EM; T4: *Fusarium* sp.; T5: *Fusarium* sp. + 1% EM; T6: 5% EM; T7: *Fusarium* sp. + chemical fungicide. Mean ± standard deviation.

3.3.2. Evaluation of Production Parameters

Production parameter data are reported in **Table 2.** The means of the parameters (bulb diameter and bulb weight) between treatments show a significant difference ($p < 0.035$ and $p < 0.041$) at the 5% threshold.

Table 2. Production parameters according to treatments.

Treatments	Dm Bulb (mm)	Pds Bulb (g)	Yields (t/ha)
T0	44.35 ± 3.1 ab	43.72 ± 13.7 ab	43.63
T01	42.88 ± 5.8 b	39.54 ± 10.8 b	39.54
T1	44.63 ± 4.2 ab	42.83 ± 16.0 ab	43.72
T2	43.39 ± 6.2 b	39.77 ± 14.0 b	39.77
T3	45.31 ± 6.8 a	43.64 ± 8.0 ab	44.09
T4	39.41 ± 7.6 c	31.01 ± 14.5 c	31.01
T5	40.23 ± 6.8 bc	33.29 ± 14.8 bc	33.29
T6	45.35 ± 5.8 a	44.09 ± 15.5 a	44.83
T7	42.81 ± 4.9 b	38.14 ± 14.5 b	38.14
Pr	0.035	0.041	

In the same column, the means of bulb diameter, bulb weight and yield followed by the same letter show no significant difference at the 5% threshold (Newman-Keuls test). Dm Bulb: bulb diameter; Pds bulb: bulb weight; T0: control; T01: *Fusarium s*p. + 2.5% EM; T1: 1% EM; T2: *Fusarium* sp. + 5% EM; T3: 2.5% EM; T4: *Fusarium* sp.; T5: *Fusarium* sp. + 1% EM; T6: 5% EM; T7: *Fusarium* sp. + chemical fungicide. Mean ± standard error.

4. Discussion

The fungal pathogen Fusarium sp. causes basal rot of onions (Allium cepa L.), with substantial loss of marketable bulbs worldwide [25]. According to [26], this pathogen originates in the field, where it may be deposited on the plant or enter the vegetable without causing disease. A recent study indicated that this pathogen can cause up to 70% of damage in nurseries, 50% of bulb losses in the field and 21.5% during the storage period, where rotten bulbs represented the greatest loss (9.1%) [7] [8] [27].

Microbiological analysis of the EM solution revealed the presence of fungi, bacteria and yeasts. Among the filamentous fungi isolated were the genera Trichoderma, Aspergillus, Penicillium and Rhizopus. The bacteria isolated on the MRS medium belong to the Bacillus family, and the yeast to the Saccharomyces cerevisiae species. This result is similar to that of [28], who defines SRMs as a mixture of several dozen strains of beneficial microorganisms composed mainly of bacteria (photosynthetic, nitrogen-fixing, acidolactic), actinomycetes, yeasts and filamentous fungi. This microbial diversity in the EM solution could be explained, on the one hand, by the use of different inputs to produce EM and, on the other, by the technique employed. Several authors have revealed that cereals and tubers such as the cassava used in this study are rich in starch (40 to

81.15% MS) [29]-[31]. This high starch content makes.

These materials are an important source of carbon and fiber for the development and growth of most isolated microorganisms. Indeed, the soil microflora is largely made up of heterotrophic organisms, *i.e.* organisms that require the supply of organic compounds, serving them, in most cases, as a source of energy and carbon [32].

Raw cow's milk also naturally contains bacteria from the acidolactic family. Added to this is the trapping technique used to capture these microorganisms. [33] showed that microorganisms are present everywhere in our environment and a single gram of soil is populated by billions of microorganisms. Consequently, bringing starch sources into contact with the soil allowed several groups of microorganisms to colonize them.

The results of the study on the ability of EMs to control fusariosis caused by Fusarium sp. in onion crops are encouraging. Disease incidence was reduced by between 23.24 and 36.52% and severity by between 30.12 and 35.08% in plants inoculated with pathogen and treated with EM This result is similar to that of [34]. Indeed, these authors showed that bacteria (*Pseudomonas fluorescens*, *Bacillus subtilis*) and the fungus *Trichoderma viride* reduced the incidence of fusariosis in onion culture by 69.5%, 59.17% and 61.8% respectively. It should also be noted that this reduction in incidence and severity was strongly dose-dependent. This ability of EMs to combat fusariosis would be linked to their functioning. According to [35], EM develops as a community in the soil, in harmony with the other native microorganisms that help them in their beneficial action. In this harmony, each family of microorganisms performs particular tasks that multiply the individual effects of each [36]-[38].

Thus, in the course of their activity, lactic acid bacteria synthesize lactic acid, which is a powerful sterilizer that suppresses pathogenic microorganisms, such as fungi of the genus Fusarium, that weaken plants and expose them to attack by other pathogens and pests [35]. Furthermore, several authors have noted that the presence of compounds with antifungal characteristics has been detected in Trichoderma cultures. Indeed, secondary metabolites from Trichoderma produce important changes in plant metabolism, acting on specific pathways involved in the synthesis of key hormones, and resistance to biotic and abiotic stress [39]-[41]. In short, like lactic acid bacteria and Trichoderma, other microorganisms secrete multiple enzymes, antibiotics and hormones that are useful to the plant against pathogens [42]. In this way, the microflora is enriched and the microbial ecosystem gradually finds a new equilibrium, transforming disease-promoting soils into disease-suppressing soils.

Experiments on the effect of EM on growth and yield parameters showed that the addition of ME gave better results in both non-inoculated and pathogen-inoculated plants. The height, number of leaves and crown diameter of the T6 treatment (5% EM) were 36.84 cm, 6 leaves and 7.58 mm respectively, compared with 36.99 cm, 6 leaves and 6.9 mm for the control. Yield was estimated at 44.33

t. ha^{-1} versus 43.63 $t{\cdot}ha^{-1}$ for the negative control (T0) and 31.01 $t{\cdot}ha^{-1}$ for the positive control (T4). These results are in agreement with those of [43]. Height, number of leaves, bulb diameter and yield were 107.2 cm, 10 leaves, 7.4 cm and 12.11 $t{\cdot}ha^{-1}$ respectively, compared with 92 cm, 6 leaves, 5.2 cm and 10.2 $t{\cdot}ha^{-1}$ for the control. These results could be explained by the presence of several beneficial microorganisms such as *Trichoderma*, lactic acid bacteria and *Saccharomycetes* present in the EM solution.

These microorganisms are known for their growth and production stimulating properties [44]. *Saccharomyces cerevisiae* has been reported to have useful stimulatory, nutritional and protective functions when applied to vegetable plants under stressful conditions due to its hormones, sugars, amino and nucleic acids, vitamins and minerals. The author noted that yeast was able to increase stimulatory growth compounds such as gibberellins, auxins and cytokinins, which act to enhance plant cell division and growth. Also, [45] reported that the Trichoderma strains studied not only produced growth regulators but also improved the delivery of nutrients from compost to roots in a way similar to the effects of mycorrhizae. As for lactic acid bacteria, they accelerate the decomposition of organic matter, thus preventing putrefaction and the appearance of unpleasant odours due to the action of harmful microorganisms [35]. In addition, filamentous fungi such as *Aspergillus*, *Rhizopus* and *Penicillium* rapidly break down organic matter to produce alcohols, esters and antimicrobial substances [35].

The results obtained showed that the higher the dose, the better the agronomic performance. These results are in line with those obtained by several authors [46]. The latter asserted that EM increased cabbage yields when the dilution was 1:500 compared with 1:1000. However, cabbage yields with the 1:1000 dilution were still 9.5% higher than in the controls.

5. Conclusion, Recommendations and Outlook

The overall aim of this study was to contribute to the improvement of onion production through the use of EM. This led us to make an inventory of the microbial genera contained in this preparation, and then to assess their ability to combat *Fusarium* sp. the onion basal rot pathogen that impacts production.

Microbiological analysis revealed the presence of *Trichoderma* sp., *Aspergillus* sp., *Rhyzopus* sp., *Penicillium* sp., Gram-positive, non-hydrogen peroxide-producing lactic acid bacteria and Saccharomyces cerevisiae (yeast). The presence of these microorganisms has proved effective against Fusarium sp, reducing its incidence and severity at a dose of 5%, as has the use of Banko-plus (a chemical fungicide). This dose reduced yield losses by 7.14%, compared with 27.59% when plants were attacked and unprotected. It also improved onion growth and yield by more than 5% when plants were not attacked at all by this pathogen.

Given the results obtained in this experiment, the use of EM at 5% can be recommended for sustainable onion production in Côte d'Ivoire.

All the results obtained open the way to other aspects that could not be explored during this experiment. These include molecular identification of the various microbial genera contained in the EM solution; testing on other pathogens and other crops, to understand the mechanism of action of EM; phenolic and biochemical assays to determine the effect of EM on the plant and harvested products.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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