

Assessment of the Effect of Biopesticides ASTOUN 50 EC and NOSTAG 50 EC on Fusarium Wilt Pathogen (*Fusarium* sp.) and on Some Agro-morphological Parameters of Common Bean (*Phaseolus vulgaris* L.)

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Abstract

Fusarium wilt caused by the fungus *Fusarium* sp. is rife in all common bean production areas in Côte d'Ivoire. It causes wilting and/or death of plants and yield losses. Chemical control is the most widely used method against this pathology. But the misuse of this method results in environmental pollution, pathogen resistance and human health problems. The search for an alternative solution to chemical control is necessary. It is in this context that this study was initiated. It aims at assessing *in vitro* and on-farm the effectiveness of the bio-pesticides ASTOUN 50 EC and NOSTAG 50 EC against common bean fusarium wilt pathogen. For this purpose, *in vitro*, five (5) doses (100, 200, 500, 1000 and 2000 µL/L) of each of the two biopesticides were tested by the method of incorporation into the PDA medium. Under natural conditions, the IC₉₀ and MIC of each bio-pesticide were tested. The results showed that the two bio-pesticides completely inhibited the mycelial growth of *Fusarium* sp. at doses of 500 µL/L. NOSTAG 50 EC showed an IC₉₀ at 300 µL/L and ASTOUN 50 EC at 400 µL/L. The biopesticides, on-farm, influenced the germination and growth parameters and reduced the wilting of the plants. In short, the 500 µL/L dose of the two biopesticides can be recommended in fusarium wilt control.

Keywords: Common bean; Fusarium wilt, *Fusarium* sp., biopesticides, Côte d'Ivoire.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food legume in the world [1, 2]. It is mainly cultivated for its grains, which are harvested when ripe [2]. Common bean grains are very high in protein (20 to 30%), starch (26 to 53%) in micro and macronutrients and in vitamins and low in lipids (1 to 3%) [3-5]. The global yield of this legume was estimated at 28,902,672 tons in 2019 [6]. Common bean cultivation is carried out in West Africa where production is estimated at over 757 thousand tons. In Côte d'Ivoire, it is produced in all regions, and is often cultivated in combination with other crops. It is appreciated by a good number of the Ivorian population. However, the domestic production of this commodity is low. It is estimated at around 43,217 tons over a surface area of 50,904 ha, for

a yield of 849 kg/ha in 2019 [6]. This low bean yield is attributable to several abiotic and biotic factors, especially fungal diseases, in particular Fusarium wilt [7, 8]. This common bean pathology is caused by *Fusarium oxysporum* f. sp. *phaseoli* and can cause losses of up to 80% in sensitive cultivars [9]. It reduces both the quantity and the quality of seed production. In order to slow down yield losses, producers resort to chemical pesticides. However, the intensive use of these synthetic products is harmful to the health of farmers, consumers and the environment [10]. Moreover, these pesticides can ease the emergence of resistant strains in pathogens [11]. Faced with this situation, it is necessary to focus the fight against pathogens on the use of natural substances [12]. Plant extract-based biopesticides are a promising way for the management of pathogens responsible for fungal diseases and

particularly fusarium wilt [13]. These biopesticides have proven their effectiveness on a wide range of pathogens and are less harmful to the health of producers and consumers [14, 15]. However, very little work mentions the use of these products against common bean fusarium wilt in Côte d'Ivoire. This study proposes to assess *in vitro* on the one hand and on the other hand under condition and on-farm the effectiveness of the biopesticides ASTOUN 50 EC and NOSTAG 50 EC against common bean fusarium wilt pathogen.

MATERIAL AND METHODS

MATERIAL

Plant material

The plant material used consisted of white and red common bean seeds purchased from stores for *in vivo* and on-farm tests. It also consisted of withered common bean plants taken from a market garden plot in the village of Ballakro in the municipality of Yamoussoukro.

Fungal material

A *Fusarium* sp. isolate used in this study was obtained from withered common bean plants.

Biopesticides

Two biopesticides (ASTOUN 50 EC whose active ingredients are Geranal and Neral) and NOSTAG 50 EC which has Thymol, Eugenol, Geranal and Neral as active ingredients) were used. These two biopesticides were formulated by the Industrial Research Unit (URI) of the University Félix Houphouët-Boigny of Abidjan Cocody, from plant extracts of the Ivorian flora.

Synthetic fungicide

The synthetic fungicide IVOIRY 80% WP (Mancozeb 800 g/kg), commonly used in market gardening in Côte d'Ivoire, served as a reference control.

METHODS

Isolation of fusarium wilts causing fungi.

The collars and stems of withered common bean plants were taken from samples of plants taken from a market garden plot in Yamoussoukro, for pathogen isolation. They were first rinsed with tap water. The removed organs were cut into approximately 0.5 cm-fragments, and then superficially disinfected by soaking in sodium hypochlorite solution (1%) for 3 min. The fragments were then rinsed three times in a row in sterile distilled water and then dried between two sterile filter papers. The dried fragments were placed in Petri dishes containing the PDA culture medium, at the rate of five (5) explants per dish. Two Petri dishes were used for each sample. The dishes were placed in the incubation room in the laboratory at a room temperature of 25 °C. After 24 to 48 hours of incubation, samples of fungus fragments from the growth fronts of the colonies

grown on the explants were taken; then subcultured onto PDA medium in new Petri dishes under a laminar flow hood. This operation was repeated until pure isolates were obtained. The fragments were collected using a sterile needle. The pathogen was identified by reference to its macroscopic characteristics and then microscopically using an identification key [16].

***In vitro* antifungal activity of biopesticides**

The method of incorporation into the PDA culture medium was the one used to assess the effectiveness of the biopesticides tested [17]. Each of the ASTOUN 50 EC and NOSTAG 50 EC biopesticides was tested at doses of 100; 200; 500; 1000; and 2000 µL/L. The synthetic fungicide (Mancozeb) was used as reference control at the single dose of 800 µL/L. The biopesticides ASTOUN 50 EC and NOSTAG 50 EC and the synthetic fungicide were incorporated into the PDA (Potato Dextrose Agar) culture medium after autoclaving. The mixture was then distributed in 90 mm-diameter Petri dishes, at the rate of 15 mL-medium in each dish. For each dose of biopesticides and synthetic fungicide, five (5) Petri dishes were used. Five Petri dishes containing non-amended PDA culture medium of biopesticides or synthetic fungicide served as control. The dishes thus prepared were inoculated with the isolate under a laminar flow hood. It consisted in removing, using a sterile punch, 6 mm-diameter mycelial discs from the growth front of the 7-day-old isolate cultures; and then placing them individually in the center of each Petri dish. The inoculated dishes were then sealed with parafilm and then incubated in the culture room at a temperature of 25 ± 2 °C. Colony mycelial growth was measured every 24 h for ten (10) days. The measurements were made along 2 perpendicular axes drawn at the base of each Petri dish, and which intersect in the middle of the explant [18]. The effect of the bio-pesticides was determined from mycelial growth inhibition rate calculated according to the formula below [19]:

$$Tmi = [(D_o - D_c)/D_o \times 100]$$

With

D_o: Mycelium inhibition rate

D_c: mean diameter of fungus mycelial growth in the control dishes;

D_c: mean diameter of fungus mycelial growth at the concentration (C) of the biopesticide /of the synthetic fungicide.

The minimum inhibitory concentration (CMI) and the concentration reducing by 90% the mycelial growth of the treated fungus were determined. They were calculated after 10 days of culture for mycelial growth. The CI₉₀ was determined with ED₅₀ plus V1.0 software [17]. At the end of the trials, the smallest concentrations of biopesticides (CMI) used at which no

development of the pathogen was obtained were noted [20].

On the tenth day of the trial, the explants from the test dishes which showed no mycelial growth were transferred to new dishes containing PDA medium without biopesticides. When the explants transferred to new culture media grew, the concentration was qualified as fungistatic, otherwise it was said to be fungicidal after seven days. The whole test was carried out twice.

On-farm test

Experimental design

The experiment was carried out in the vegetable patch of the University Peleforo GON COULIBALY (UPGC). It was carried out according to a Fisher block design at a rate of 3 blocks with 6 treatments. The blocks were separated from each other by 1 m. The elementary plot or treatment covered a surface area of 4.5 m² (3 m x 1.5 m), and a distance of 0.5 m separated elementary plots of the same block and 1 m for 2 neighboring blocks. Each elementary plot consisted of two seeding rows each comprising four pockets with 0.5 m-spacing between seeding lines and 0.7 m between the pockets of the same row. The 6 treatments are composed as follows: T0: control; T1: untreated inoculated seeds; TA1: ASTOUN 50 EC CI90 (400 µL / L); TA2: ASTOUN 50 EC CMI (500 µL/L); TN1: NOSTAG 50 EC CI90 (300 µL/L) and TN2: NOSTAG 50 EC CMI (500 µL/L). The total surface area of the experimental plot was (11.5 m x 10 m), that is, 115 m².

Sowing and seed inoculation

The physically sound and undamaged seeds of the white and red common bean varieties were used. These seeds were soaked in solutions at the concentration of 400 and 500 µL/L of the biopesticides ASTOUN 50 EC and 300 and 500 µL/L of NOSTAG 50 EC. The control seeds were soaked in a volume of sterile distilled water. The duration of the different soaks was 8 hours. Sowing was carried out on the 18 elementary plots at the rate of 2 seeds per pocket and at a depth of 5 cm. The sowing points on the same line were 70 cm apart, that is, 8 pockets per elementary plot. Inoculation with the fungal isolate was done approximately 2 hours after sowing. It consisted of sprinkling 5 drops of the inoculum solution (1x10⁶ spores / mL) in the pockets of the treatments except those of the control. The drops were applied in the pocket so that the seed point was wet for contamination.

Parameters measured

Germination time and rate

It concerned the date of seedling emergence. Emergence and sowing dates were noted. These dates were used to determine the emergence time (or

germination period). On-farm, common bean seed germination rate was assessed by making the ratio between the germinated seeds and the total number of seeds sown. This rate was determined from the following formula [21].

$$Tg (\%) = \frac{Nge}{Ntg} \times 100$$

Where

Tg is the rate of germinated seeds;

Nge: Number of germinated seeds and Ntg total number of seeds sown.

Agromorphological parameters

The agro-morphological parameters considered were the height and the total number of leaves of the plants. For this purpose, 04 plants of each treatment per block, that is, a total of 12 plants per treatment, were selected for the measurements. These were done every week for five weeks.

Phytosanitary parameter

The parameter mainly focused on the incidence of fusarium wilt on common bean plants. The number of withered common bean plants in all the common bean plants assessed was used to assess the incidence of fusarium wilt according to the formula of James [22].

$$If (\%) = \frac{NPf}{NPE} \times 100 \quad \text{with IF (\%) Wilt incidence rate; NPf number of withered plants and NPE Total number of plants assessed.}$$

STATISTICAL ANALYSES

The data collected was analyzed using STATISTICA version 7.1 software. One-factor analyses of variance (ANOVA 1) were performed to obtain the means of the different parameters. When a significant difference was observed, the Newman-Keuls test at 5% (0.05) threshold was performed to classify the means into homogeneous groups.

RESULTS AND DISCUSSION

Results

Effects of biopesticides on isolate mycelial growth

Fusarium sp. sensitivity varied depending on the biopesticide used. It was also different depending on the concentrations used and the incubation time. Thus, concerning the biopesticide ASTOUN 50 EC, the doses of 100 and 200 µL/L reduced mycelial growth on the 2nd and 10th day respectively by 54.35 ± 3.58 and 100% to 26.24 ± 6.36 and 79.52 ± 1.54% (Figure 1). As for the doses of 500, 1000 and 2000 µL/L, the reduction rate was 100% for each during the same period. The single dose of the synthetic fungicide IVORY 80% WP (Mancozeb 800 g/kg), also completely reduced *Fusarium* sp. mycelial growth throughout the

experiment. Moreover, apart from the 100 $\mu\text{L/L}$ dose, the four other doses of ASTOUN 50 EC induced a reduction of more than 50% in mycelial growth during the test (Figure 1).

For the biopesticide NOSTAG 50 EC, the reduction rate in mycelial growth also varied depending on concentration and time (Figure 2). All doses of this biopesticide strongly reduced mycelial growth. Thus, the doses of 100 $\mu\text{L/L}$ and 200 $\mu\text{L/L}$ strongly inhibited growth by more than 60% from the 2nd to the 10th day. As for the doses of 500, 1000 and 2000 $\mu\text{L/L}$, the

reduction rate was 100% throughout the duration of the test (Figure 2). The average reduction rate in *Fusarium* sp. mycelial growth by the two biopesticides was also determined (Figure 3). It indicated that the biopesticides ASTOUN 50 EC and NOSTAG 50 EC totally inhibited mycelial growth from the dose of 500 $\mu\text{L/L}$. This reduction in mycelial growth at the dose of 100 $\mu\text{L/L}$ was 36.47% for the bio-pesticide ASTOUN 50 EC and 66.47% for NOSTAG 50 EC. At the dose of 200 $\mu\text{L/L}$, the reduction in mycelial growth was 78.58% for ASTOUN 50 EC and 82.7% for NOSTAG 50 EC (Figure 3).

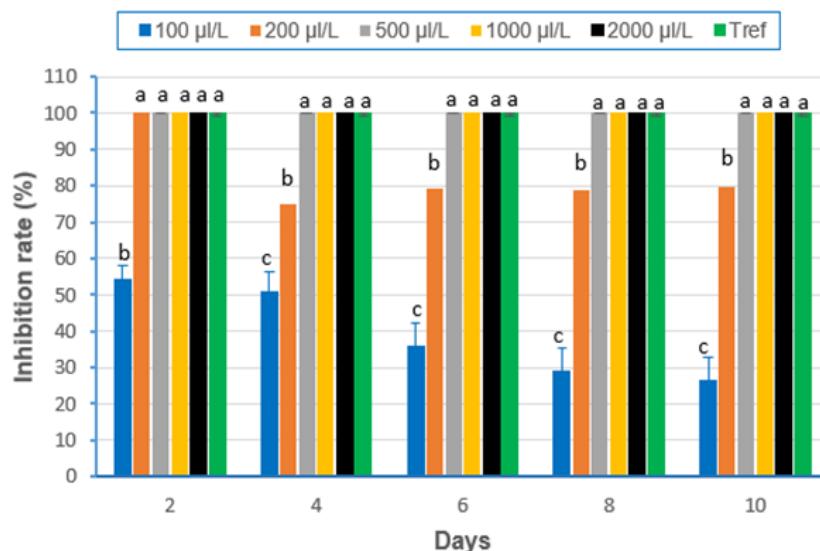


Fig-1: Reduction rate in *Fusarium* sp. mycelial growth by the biopesticide ASTOUN 50 EC compared to the synthetic fungicide Tref depending on time.

Significant differences ($p < 0.05$) among averages are indicated by letters above histogram bar. Where the letters are the same, there is no significant difference among different inhibition rates.

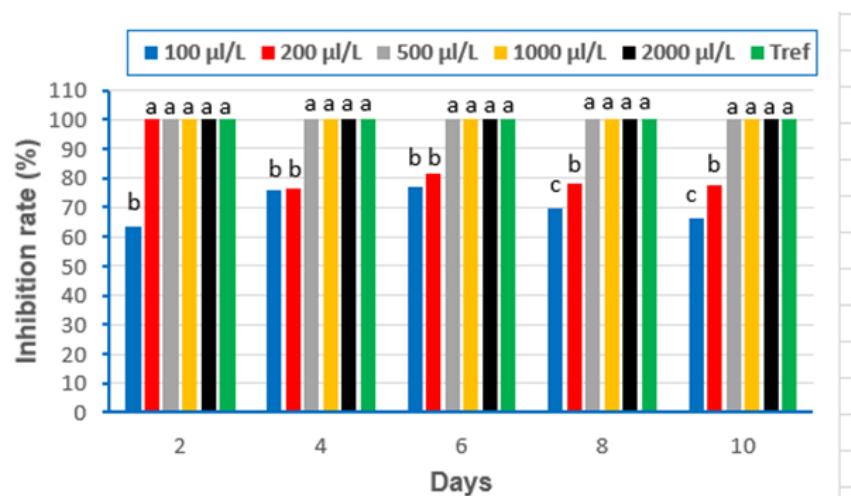


Fig-2: Reduction rate in *Fusarium* sp. mycelial growth by the biopesticide NOSTAG 50 EC compared to the synthetic fungicide Tref depending on time.

Significant differences ($p < 0.05$) among averages are indicated by letters above histogram bar. Where the letters are the same, there is no significant difference among different inhibition rates.

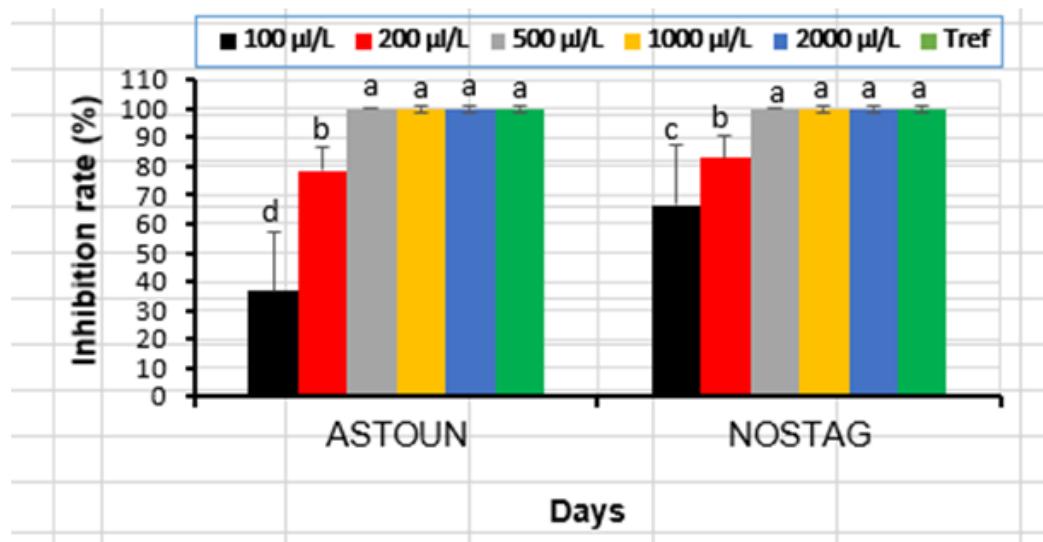


Fig-3: Average reduction rate in *Fusarium* sp. mycelial growth by the biopesticides ASTOUN 50 EC, NOSTAG 50 EC compared to the Synthetic fungicide on the tenth day of incubation.

Significant differences ($p < 0.05$) among averages are indicated by letters above histogram bar. Where the letters are the same, there is no significant difference among different inhibition rates.

The smallest dose at which the two biopesticides (MIC) completely inhibited *Fusarium* sp. mycelial growth was 500 µL/L. Thus, the concentration of ASTOUN 50 EC which reduced mycelial growth by 90% (CI_{90}) was 400 µL/L. As for the biopesticide NOSTAG 50 EC, the IC_{90} was 300 µL/L. The mycelial explants of the PDA culture media amended with the biopesticides ASTOUN 50 EC and NOSTAG 50 EC at doses greater than or equal to 500 µL/L, could not grow after 7 days following the transfer to a non-amended PDA medium. These biopesticides had a fungicidal effect on mycelial growth at doses of 500; 1000 and 2000 µL/L.

On-farm test

Germination rate

Table I shows the seed germination rate per treatment depending on time and the average germination rate on the 7th day after sowing. The analyses showed that treatments TA2, TN1 and TN2 induced a high rate of seed germination throughout the experiment. These rates ranged from 75% to 100% from the 3rd to 5th day of the experiment. In contrast, the uninoculated and untreated inoculated controls showed the lowest germination rates (Table I). Treatments TA2, TN1 and TN2 showed a mean germination rate statistically different from those of the controls ($p = 0.015$).

Plant height

Figure 4 shows the increase in plant height per treatment depending on time. It indicates that the height

of the plants treated as the control varied from the 1st to the last week with an average ranging from 7.46 to 58.25 cm. The greatest height was obtained with the seedlings of the seeds of the treatments with Biopesticide NOSTAG 50 EC, especially TN2. The latter gave average heights of 16.46 ± 1.15 ; 21.5 ± 1.28 ; 28.83 ± 1.28 and 58.25 ± 1.28 cm respectively in the 2nd, 3rd, 4th and 5th week. As for the plants of treatments T1 and the control, they showed the lowest average heights (Figure 4).

Total number of plant leaves

Analysis of the average number of leaves in **Figure 5** showed that treatment TN2 plants produced the highest average number of leaves with averages of 9.16 ± 1.21 ; 15.58 ± 1.21 ; 35.66 ± 1.21 ; 38.91 ± 1.21 and 43.83 ± 1.21 during the 1st, 2nd, 3rd, 4th and 5th week, respectively. As for the plants of treatments T1 (5.75 ± 1.21 ; 6.58 ± 1.21 ; 9.66 ± 1.21 ; 13.16 ± 1.21 and 17.5 ± 1.21), as well as the control (6.66 ± 1.21 ; 9.66 ± 1.21 ; 10.83 ± 1.21 , 13.83 ± 1.21 and 19 ± 1.21) produced the lowest average number of leaves (Figure 5).

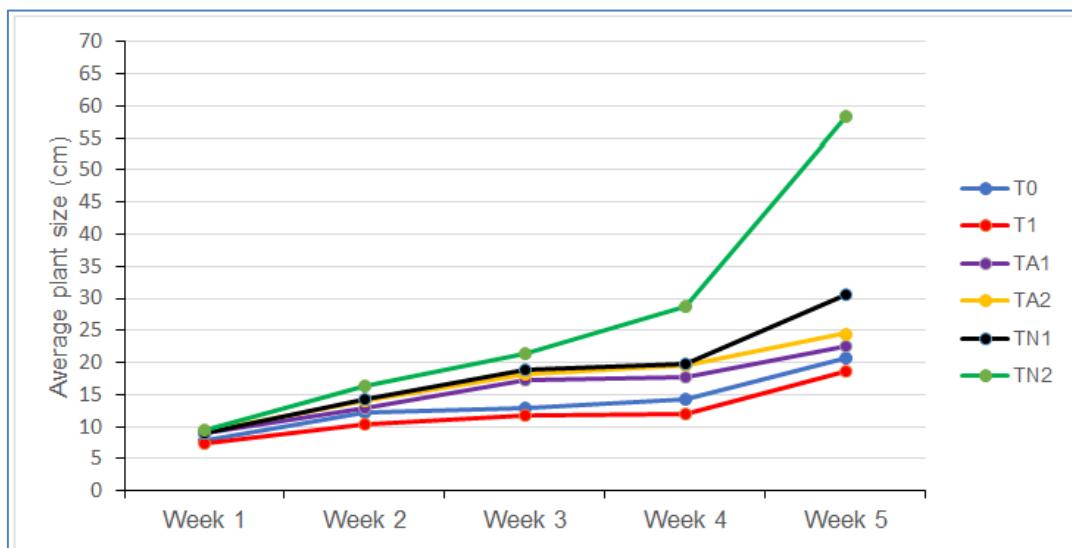
Incidence of plants wilt

Analysis of **Table II** indicates that the treatments had a highly significant influence ($p=0.001$) on plant wilting incidence. The analyses showed that the plants from treatments T1 as well as the control showed a high incidence rate (9.99 and 5.83%) compared to the plants from treatments TN2, TN1, TA1 and TA2.

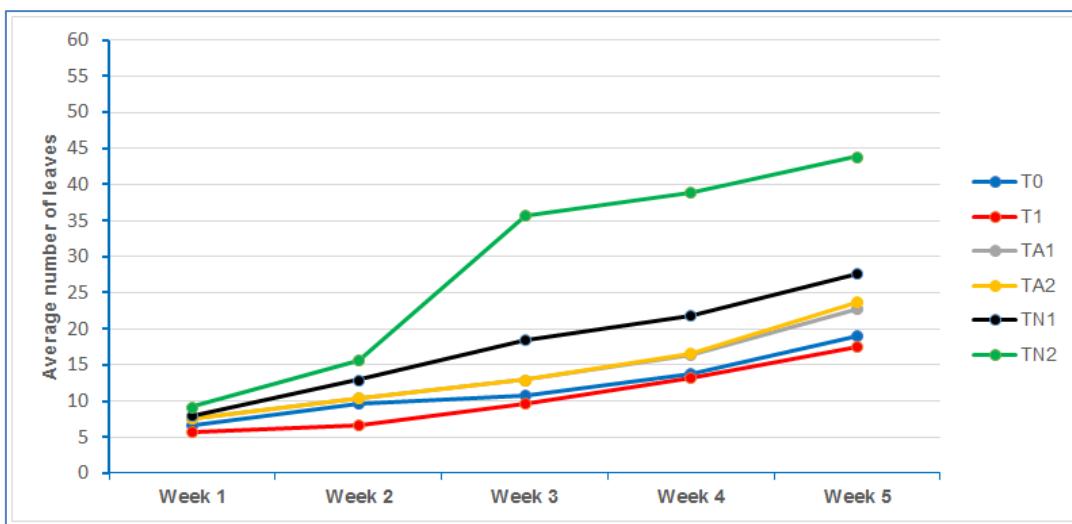
Table-1: Germination rate of common bean seeds depending on time and the Average rate on the 7th day after sowing.

Treatments	Time (Days)					Average germination rate (%)
	3	4	5	6	7	
T0	50	50	75	87.5	100	72.5 ± 8.75 bc
T1	37.50	37.50	62.5	87.5	100	65 ± 8.75 c
TA1 (400 µl/l)	62.5	62.5	100	100	100	85 ± 8.75 ab
TA2 (500 µl/l)	75	75	100	100	100	90 ± 8.75 a
TN1 (300 µl/l)	75	75	100	100	100	90 ± 8.75 a
TN2 (500 µl/l)	75	75	100	100	100	90 ± 8.75 a

The means assigned the same alphabetical letters are not significantly different at 5% threshold according to the Newman Keuls test.

**Fig-4: Average height of plants per treatment depending time**

T0: control; **T1:** untreated inoculated seeds; **TA1:** ASTOUN (400 µl/L); **TA2:** ASTOUN (500 µl/L); **TN1:** NOSTAG (300 µl/L); **TN2:** NOSTAG (500 µl/L)

**Fig-5: Average number of leaves per treatment depending on time**

T0: control; **T1:** untreated inoculated seeds; **TA1:** ASTOUN (400 µl/L); **TA2:** ASTOUN (500 µl/L); **TN1:** NOSTAG (300 µl/L); **TN2:** NOSTAG (500 µl/L)

Table-II: Incidence rate of plant wilting depending on treatments

Treatments	Incidence rate (%)
T0	<i>9.99±1.59a</i>
T1	<i>5.83±1.59ab</i>
TA1	<i>1.66±1.59b</i>
TA2	<i>1.66±1.59b</i>
TN1	<i>0.83±1.59b</i>
TN2	<i>0.00±1.59b</i>

T0: control; **T1:** untreated inoculated seeds; **TA1:** ASTOUN (400 µL/L); **TA2:** ASTOUN (500 µL/L); **TN1:** NOSTAG (300 µL/L); **TN2:** NOSTAG (500 µL/L)

The rates assigned the same alphabetical letters are not significantly different at 5% threshold with the Newman Keuls test.

DISCUSSION

The fungitoxicity of the biopesticides ASTOUN 50 EC and NOSTAG 50 EC was assessed under *in vitro* culture conditions and on-farm. Regarding *in vitro* culture, the two biopesticides totally reduced *Fusarium* sp. mycelial growth from 500 µL/L. This result reveals that these biopesticides are endowed with a strong antifungal activity against the pathogen *Fusarium* sp. Our results are in agreement with those reported by the work of Fofana *et al.*, [15]. These authors have proved that the plant extract-based biofungicides NECO 50 EC, ASTOUN 50 EC and FERCA 50 EC exerted a strong inhibitory activity *in vitro* on the growth of *Phytophtora palmivora*, the agent responsible for brown rot in cocoa pods. The ability of the two biopesticides to inhibit *Fusarium* sp. mycelial growth in this study would be due to their components. Indeed, the biopesticide ASTOUN 50 EC is composed of Geranial, Myrcene and Neral. As for the biopesticide NOSTAG 50 EC, it consists of Thymol – Eugenol and Geranial-Néral. Geranial and Neral are two isomers that make up Citral. The antifungal properties of this constituent (Citral) are proven by several previous works. The work of Venturinic *et al.* [23] demonstrated the strong antifungal activity of Citral, Eugenol and Thymol against *Penicillium expansum* agents responsible for apple rot. Kassi *et al.*, [17] also reported the effectiveness of the essential oil *Ocimum gratissimum*, mainly composed of Thymol, against isolates of *Fusarium oxysporum* f. sp. *lycopersici*, from three regions of Côte d'Ivoire. However, these results contrast with those of Kassi *et al.*, [17] that did not determine an MIC, while ours showed an MIC of 500 µL/L. This strong activity of our biopesticides compared to the essential oil of *Ocimum gratissimum* L. is attributable to the combination of active ingredients that compose them. The antifungal activities of these constituents of essential oils could be attributed to their lipophilic character, which interacts with the cell wall, then penetrates the plasma membrane of the fungal parasite, which becomes permeable. This results in the leakage of cytoplasmic constituents, eventually leading to the death of the parasite [24]. These two biopesticides were fungitoxic at the

dose of 500 µL/L, because from this dose, no regrowth of the pathogen was observed. Similar results have been reported by Soro *et al.*, [25] during their work on the comparative activities of the essential oil of *Ocimum* and two synthetic fungicides. The biopesticide NOSTAG 50 EC had a greater effect than that of ASTOUN 50 EC, this result is essentially linked to the composition of these two biopesticides. These two biopesticides showed inhibitory activities similar to that of the synthetic fungicide IVORY 80% WP (Mancozeb 800 g/kg) at slightly lower doses. They can therefore be used instead of this pesticide in a common bean fusarium wilt control strategy.

On-farm, the results indicate that the doses of the biopesticides used eased common bean seed germination compared to the inoculated untreated seeds and the uninoculated control. Our results are contrary to those reported by Ait Ialeff *et al.*, [26]. These authors found that a bioproduct formulated with essential oil extracted from *Cupressus arizonica* did not influence the germination of durum wheat (*Triticum durum*). The same observation was made a little earlier by Kritzinger *et al.*, [27] during their work on the effect of essential oils on storage fungi, germination and emergence of cowpea seeds. This difference in results could be attributed to the seeds and products used in each case. This high germination rate of common bean seeds treated with these biopesticides indicates that the biopesticides would induce the breaking of dormancy in these seeds by activating the reactions of hydrolysis of reserves and the enzymatic activities which result in seed germination [28]. Furthermore, the plants from the treated seeds showed great heights with the highest number of leaves. The plants from the 500 µL/L dose of NOSTAG 50 EC produced the highest number of leaves and were the greatest in height. This result contrasts with those of Ait Ialeff *et al.*, [26]. They found in their work that only the smallest dose of *Cupressus arizonica* essential oil stimulated wheat root growth. Moreover, the plants resulting from seeds treated with the biopesticides ASTOUN 50 EC and NOSTAG 50 EC at different doses showed a low incidence of wilting compared to the untreated inoculated control and the control. Thus, these biopesticides, through seed treatment, prevented the spread of *Fusarium* sp. and the wilting or death of plants. This would be justified by the effectiveness of the active ingredients in these products.

The 500 µL dose of NOSTAG 50 EC stimulated germination and plant growth

CONCLUSION

Mycelial growth inhibition tests carried out with the bio-pesticides ASTOUN 50 EC and NOSTAG 50 EC at different doses have demonstrated their inhibitory power on *Fusarium* sp. These biopesticides completely inhibited *Fusarium* sp. mycelial growth from the dose of 500 µL As for the synthetic fungicide, it has proven to be effective at the single dose of 800 µL regarding on-farm test, the biopesticides eased seed germination and plant growth. They would be considered biostimulants. These two biopesticides can be used in an integrated *Fusarium* wilt control strategy. However, it would be important to assess the effect of these biopesticides on the growth and agro-morphological parameters and the components of the yield of common bean and other crops in peasant environments with a view to their popularization.

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