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Evaluation of the effect of two biofertilizers (*Azolla* spp and *Daldinia concentrica*) on the agro-morphological parameters of green soybean (*Vignata radiata*) in Daloa (Center-West, Côte d'Ivoire)

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Abstract

Soybean cultivation, despite its beneficial potential and favorable conditions, is not valorized in Côte d'Ivoire. The production of soybeans in Côte d'Ivoire was 1000 tons in 2013. The objective of this study is to promote the use of biofertilizers (*Daldinia concentrica* and *Azolla* spp) to improve green soybean production in Côte d'Ivoire. The experimental set-up used is a randomized Fisher block, with five replications and five treatments: control, *Azolla* spp, *Daldinia concentrica, Azolla* spp and *Daldinia concentrica* combination and NPK 12-22-22 fertilizer. The results obtained showed that the effect of the biofertilizer associating *Azolla* spp and *Daldinia concentrica* on soybean was expressed, respectively, through the growth by better effects on the height of the stem, the diameter at the collar, the number of leaves, the number of ramification and the length of the root. It should be noted that the treatment combining *Azolla* spp. and *Daldinia concentrica* was the best biofertilizer used in this study.

Keywords: *Azolla spp, Daldinia concentrica, Growth, Production, Soybean, Daloa*

1. Introduction

The Ivorian economy has long been based on agriculture. This agriculture has been productive thanks to the large availability of fertile, cultivable land and adequate rainfall. These assets, combined with a favorable climate, have allowed Côte d'Ivoire to exploit a wide range of crop production (Sangaré *et al*., 2009). However, this agriculture is essentially based on industrial crops (coffee, cocoa, cotton, rubber, oil palm, cashew nuts,

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pineapple, mango, papaya, sweet banana, sugarcane, etc.). These industrial crops are favored to the detriment of food crops such as protein crops and vegetables. Also, food crops constitute the primary resources for staple food in developing countries and more particularly in Côte d'Ivoire (Didinger and Thompson, 2021; and Akah *et al*., 2021). On the other hand, legumes are of undeniable interest for the fertilization of agricultural soils because of their nitrogen fixing capacity. All these interests would be capitalized if this national agriculture was not faced with enormous environmental difficulties. These include climate change, land misuse, deforestation and especially the uncontrolled application of plant protection products (Tandzi and Mutengwa 2020; and Suko and Ernoiz, 2021).

However, the use of chemical fertilizers not only pollutes groundwater by emitting harmful gases but also remains expensive and inaccessible to farmers (Useni *et al*., 2013; and Oluwaseun *et al*., 2021). Chemical fertilizers need to be replaced by natural and organic fertilizers (biofertilizers) that can play a key role in environmental conservation. Biofertilizers improve the quantitative and qualitative characteristics of many plants (Doifode, 2021; Nosheen *et al*., 2021; and Oluwaseun *et al*., 2021). According to Yosefi *et al*. (2011) all these problems lead to serious consequences such as modification of ecosystem stability, annihilation of underground fauna (microorganisms), modification of hydrogen potential (pH) which considerably impact the growth, development and yield of crops (Yanan *et al*., 2020; and Heena *et al*., 2021).

Faced with all these constraints, the use of biofertilizers in agriculture is one of the best solutions taking into account the respect of the environment (Nosheen *et al*., 2021).

It is in this context that this work was initiated to evaluate the effect of two (*Azolla* spp and *Daldinia concentrica*) on soybean growth parameters in Côte d'Ivoire.

2. Material and methods

2.1. Presentation of the study area

This study was conducted on the experimental plot of the University Jean Lorougnon Guédé in Daloa. The city of Daloa is located in the Upper Sassandra region (Central-West of Côte d'Ivoire) between 6° and 7° North latitude and 7° and 8° West longitude. This region has an area of 15200 km² (Ba, 2014). The climate has four seasons including the major rainy season from April to mid-July, the minor dry season from mid-July to mid-September, the minor rainy season from mid-September to mid-November, and the major dry season from December to March with temperatures ranging from 24.65°C to 27.75°C on average. The average rainfall, temperature and humidity during the test periods from May to August 2021 are respectively: 142.81 mm; 26.42°C and 83.7. The soils in the region are mostly ferralitic (typical). They are generally very deep with a high organic matter content. They are ferralitic soils of granitic origin with medium to low desaturation. They have good agricultural suitability and are suitable for all crop types (Koffie and Kra, 2013).

2.2. Material

The material used in this study is of various natures. They are plant material, fungal material and chemical material.

2.3. Plant material

The plant material used in this study consists of plants from green soybean (*Vigna radiata*) seeds obtained on the market of Daloa and an aquatic fern which is *Azolla spp.*

2.4. Materials fungus

A species of non-edible mushroom that is *Daldinia concentrica.* This fungal species is a member of the saprophytic Ascomycetes family that grows on dead wood. This mushroom is used a lot in traditional medicine for its therapeutic virtues.

2.5. Chemical material

The chemical fertilizer used was the mineral fertilizer NPK 12-22-22. It is the most used fertilizer by the farmers in the soybean crop.

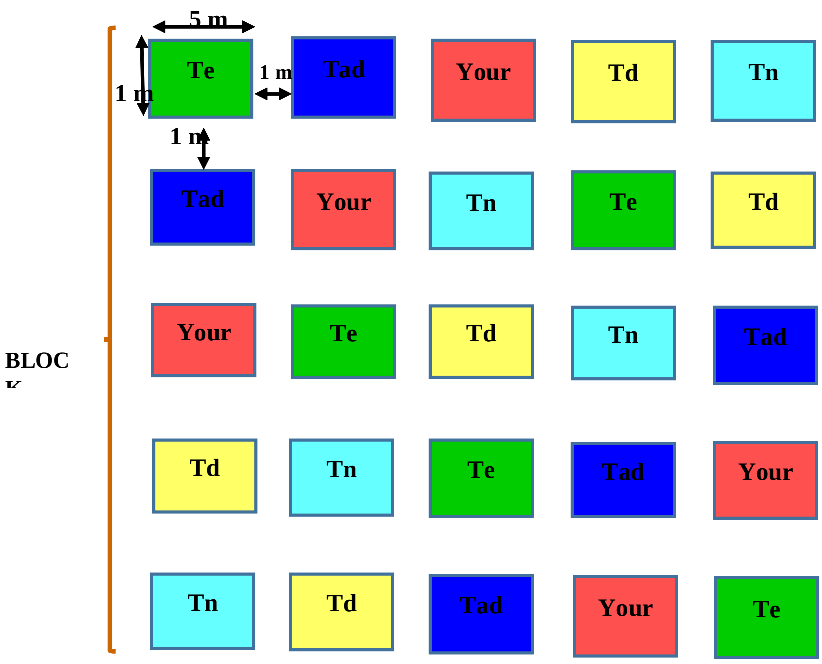
Figure 1: The material used in this work; A: *Daldinia concentrica*; B: *Azolla* spp.

2.6. Methodology

The methodological approach adopted to carry out this study includes several phases which are: the setting up of the experimental device, the collection of the agro-morphological data and finally, the statistical analysis of the collected data.

2.7. Experimental device

The experimental design was established according to the randomized block design. This set-up is made up of five (5) treatments with five (5) repetitions, i.e., a total of 25 unit plots of dimension 5 m^2 (5m x 1 m) each. These treatments are randomly distributed in each block.



Note: Te: Control treatment; **Ta**: Treatment with *Azolla* spp; **Td**: Treatment with *Daldinia concentrica*; **Tad**: Treatment with *Azolla* spp + *Daldinia concentrica*; **Tn**: Treatment with NPK

Figure 2: The experimental setup

2.8. Setting up the culture

The establishment of the crop began with a preparation of the soil with a hoe that was used for clearing. This preparation was followed by the preparation of the 50 to 60 cm wide ridges. Finally, stacks were made in rows, respecting a distance of 50 cm between rows and 20 cm between stacks (10 stacks per ridge). Each ridge is made up of two lines.

2.9. Preparation of biofertilizers

2.9.1. Culture medium of Azolla *spp.*

The preparation of the culture medium consists in the use of a rectangular germoir in covered with a black plastic bag inside to avoid water leakage. The water and the droppings were added in the germoir (100 liters of water). A mixture was carried out thereafter, to obtain a homogeneous solution. Finally the Azolla was added to the obtained solution, the whole was covered with a mosquito net to avoid the entry of the pests. The culture was left to incubate for two weeks to promote the production of Azolla.

2.10. Preparation of **Daldinia concentrica** *powder*

The *Daldinia concentrica* mushroom was dried for 10 days. Once the mushroom was dry, it was ground into a powder. This powder was weighed for application in the pits before semi.

2.11. Seed selection and sowing

Seeds were subjected to the flotation test to select healthy and mature seeds. The seeds were placed in a jar containing plain water. Floating seeds were removed to keep only those that remained at the bottom. Semi-drying was done one week after the application of the different fertilizers. It was done at a rate of two seeds per poquet with a spacing of 50 x 20 cm and at a depth of 3 cm. A demariage was carried out to finally leave only one plant by poquet.

2.12. Application of fertilizers

Organic and chemical fertilizers were first applied to the pits one week before planting. The application of *Daldinia concentrica* fertilizer and NPK fertilizer (12-22-22) was done with an amount of 10 g per dedicated poquet. The second application of biofertilizers was made one week after sowing and repeated (at an interval of 7 days) over a period of one month. NPK fertilizer (12-22-22) was applied every two weeks (14 days) over a period of one month; the first application started two weeks after sowing.

3. Data collection

All data were collected every week from the date of sowing to the appearance of flower buds. They concerned the height of the stem, the diameter at the collar, the number of leaves.

4. Observation and measurement of parameters

Measurements were taken from five (5) plants in each plot taken at random for each treatment. The number of leaves was obtained by simple counting. Stem height was determined by measuring the distance of the main stem from the collar to the outermost leaf. Diameter at the collar was measured from the circumference of the main stem, using a tape measure and a caliper.

5. Statistical analysis

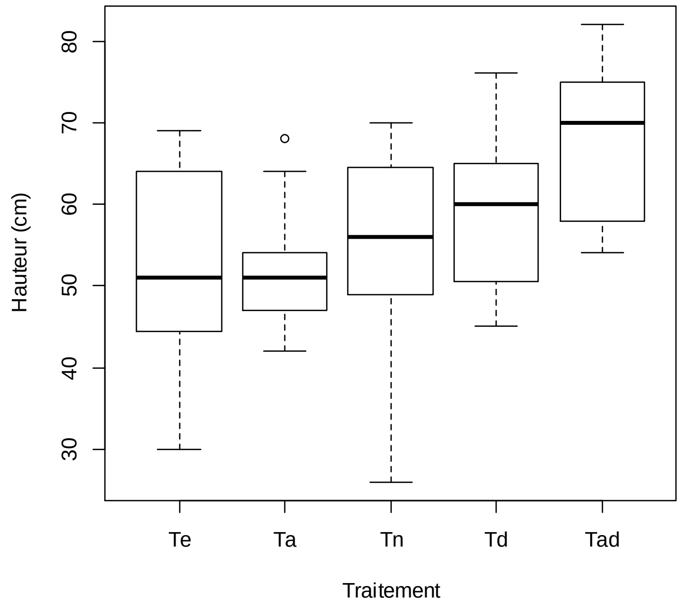
Data were subjected to statistical analysis using R software and STATISTICA 7.1 software. The analysis of varianManuscript Submissionce with one (1) factor (ANOVA). The comparison of means according to the LSD Fischer test at the 5% threshold was carried out, followed by tests of significance and homogeneity.

6. Results and discussion

6.1. Effect of treatments on at stem height

Figure 3 shows the results of the different treatments on soybean stem height. All the treatments tested, significantly influenced the height of the stem as the $p = 0.0004$. However, the treatment combining *Azolla spp* and *Daldinia concentrica* (Tad) as biofertilizer, recorded the highest values (67.6 cm) compared to the control treatment.

Three distinct groups were observed: The first group consists of Te (51.86), Ta (51.93) and Tn (54.13). The second group consists of Td (58.66) and the third group consists of Tad (67.6). The tested treatments Td (58.66) and Tad (67.6) significantly influenced stem height.

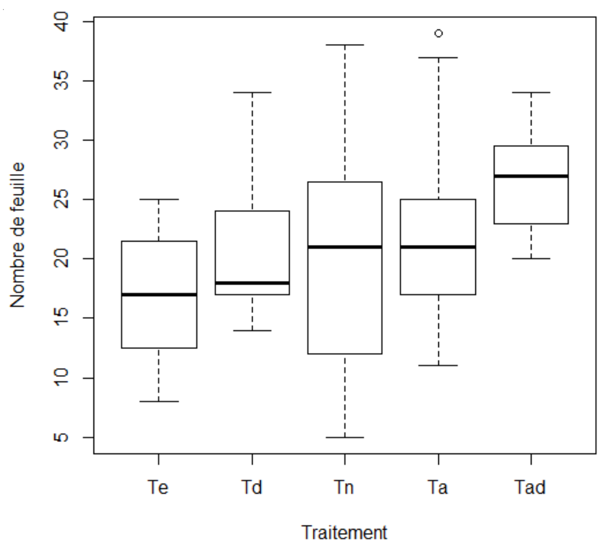


Note: Te: Control treatment; **Ta**: Treatment with *Azolla* spp; **Td**: Treatment with *Daldinia concentrica*; **Tad**: Treatment with *Azolla* spp + *Daldinia concentrica*; **Tn**: Treatment with NPK

Figure 3: Influence of treatments on soybean stem height

6.2. Effect of treatments on the number of leaves

Figure 4 shows the results of the different treatments on the number of soybean leaves. All the treatments tested, significantly influenced all the parameters evaluated. The treatment combining *Azolla* spp and *Daldinia*



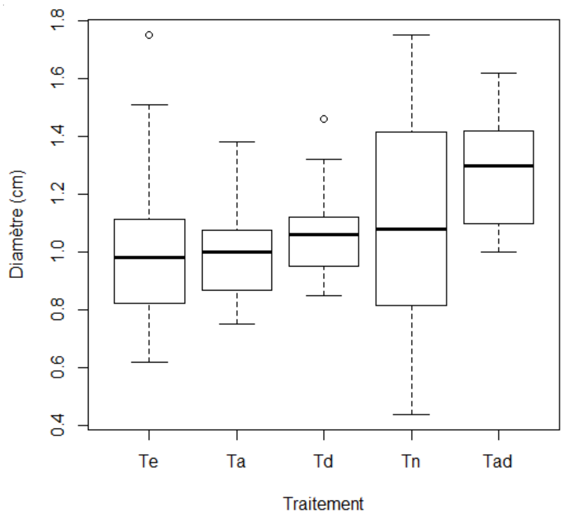
Note: Te: Control treatment; **Ta**: Treatment with *Azolla* spp; **Td**: Treatment with *Daldinia concentrica*; **Tad**: Treatment with *Azolla* spp + *Daldinia concentrica*; **Tn**: Treatment with NPK

Figure 4: Influence of treatments on the number of soybean leaves

concentrica (Tad) recorded the highest values (26.8). The lowest value (17.48) was obtained with the control treatment. Four groups can be distinguished for the number of leaves: The first group includes the control treatment (17.48). The second group consists of the Tn (20.66) and Td (21) treatments. The third group consists of the Ta treatment (23) and the fourth group consists of the Tad treatment (26).

6.3. Effect of treatments on crown diameter

Figure 5 shows the results of the different treatments on soybean crown diameter. These results revealed that all the treatments studied had a significant effect on the diameter at the crown because *p* less than 0.05 (*p* = 0.042). Three groups can be distinguished: The first group consists of Te (1), Ta (1.02) and Td (1.04), The second group includes Tn treatment and the third group consists of Tad treatment. The tested treatments Tn (1.16) and Tad (1.28) significantly influenced the diameter at the neck.



Note: Te: Control treatment; **Ta**: Treatment with *Azolla* spp; **Td**: Treatment with *Daldinia concentrica*; **Tad**: Treatment with *Azolla* spp + *Daldinia concentrica*; **Tn**: Treatment with NPK

Figure 5: Influence of treatments on crown diameter

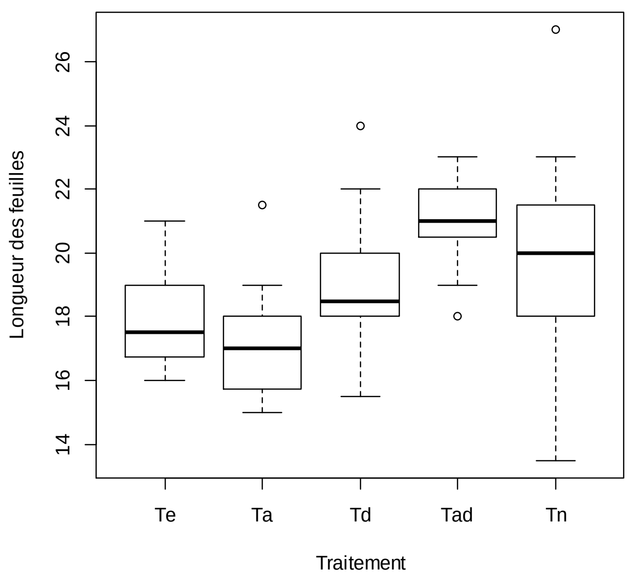


Figure 6: Influence of treatments on leaf length

6.4. Effect of treatments on leaf length

Figure 6 shows the results of the different treatments on soybean leaf length. These results showed that all the treatments studied all have a significant effect on leaf length as *p* less than 0.05 (*p* = 0.0001). Three distinct groups are annotated:

The first group consists of Ta (17.13) and Td (1.04), Te (1). The second group consists of Tn treatment and the third group consists of Tad treatment. The tested treatments Tn (1.16) and Tad (1.28) significantly influenced the diameter at the neck.

7. Discussion

In this study, the treatment combining *Azolla spp* and *Daldinia concentrica* showed the best effects on soybean growth parameters including stem height, crown diameter, number of leaves. These results are explained by the fact that the combination of *Azolla spp* and *Daldinia concentrica*, would have more mineral elements necessary for the growth and development of soybean plants. A similar result was observed by Adiprasetyo *et al*. (2014) where the multi-microbial biofertilizer (PGPR) increased plant growth (to increase height and number of leaves) of oil palms compared to chemical fertilizer alone.

Nutrients made sufficiently available over time in the soil are efficiently used by crop plants (Kouamé *et al*., 2021).

The combination of these two fertilizers stimulated plant growth by creating a favorable root environment, not only for nutrient uptake, but also for atmospheric nitrogen fixation. The combined treatment of Daldinia and Azolla (Tad) 1.28 promoted better growth of soybean leaves. According to Ojetayo *et al.* (2011), Akande *et al.* (2003), and Olaniyi *et al*. (2010), these organic materials play an important role in the soil and are favorable to the growth of microorganisms that induce an activation of the solubilization of nutrients. For Kouassi *et al*. (2019), the use of organic manure in case of soil organic matter deficiency is necessary. It allows to filling the water deficit during the production phase.

The mineral fertilizer NPK (12-22-22) gave slightly less results than the organic fertilizer based on *Azolla sp.* and *Daldinia concentrica*. In fact, mineral fertilizers are substances produced by the chemical industry or by the exploitation of natural deposits. These mineral elements put at the disposal of the plant are used until they are broken. They are therefore not available to the plant continuously and their deficit in the soil influences the end of the development cycle (Kouamé *et* al., 2021).

The work of Mulaji (2011) shows the superiority of the green biomass of *Tithonia diversifolia* compared to mineral fertilizers. Mineral fertilizers, despite their rapid release of mineral elements, would be linked to the improvement of soil properties coupled with the supply of mineral elements. Indeed, mineral fertilizers play the trophic role while organic amendments play the trophic role and improve at the same time the properties of the soil

Kouamé *et al.* (2021) noted that the mineral fertilizer performed worse than chicken droppings but far better than the sawdust-based organic fertilizer, which performed about the same as the control without fertilizer.

The work of Dibi *et al.* (2021) on yam reported that the combined use of poultry droppings and mineral fertilizer significantly improved growth compared to the application of each fertilizer separately.

Kouassi *et al.* (2021) in their work proved that mineral fertilizer had the best effects on soybean growth parameters including height, crown diameter and vigor index of plants in early growth. This result is explained by the fact that the mineral fertilizer would have directly available mineral elements. These minerals would have favored the rapid growth of the plants.

8. Conclusion

The general objective of this study was to promote the use of biofertilizers (*Daldinia concentrica* and *Azolla spp*) for the improvement of green soybean production in Côte d'Ivoire. In this study, the results showed that the combination of biofertilizers *Azolla spp* and *Daldinia concentrica* has a beneficial impact on rapid plant growth. Also, the biofertilizer combining *Azolla spp* and *Daldinia concentrica* had a positive effect on growth parameters (stem height, crown diameter, number of leaves). Thus, it should be noted that the treatment combining *Azolla spp* and *Daldinia concentrica* was the best biofertilizer used in this study. On the other hand, the treatment consisting only of *Azolla spp* as biofertilizer did not express itself beneficially to the studied soybean accession.

It should be noted that biofertilizers are widely used as an ecological approach to minimize the use of chemical fertilizers, improve soil fertility status and for the improvement of crop production through their biological activity in the rhizosphere.

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