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RESEARCH ARTICLE

EVALUATION OF TWO BIOFERTILIZERS BASED ON *AZOLLA FILICULOIDES* AND *AZOLLA CAROLINIANA* IN CABBAGE (*BRASSICA OLERACEA*) PRODUCTION IN DALOA

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ABSTRACT

The high cost of mineral fertilizers and their low accessibility to farmers is a limiting factor in cabbage cultivation. This crop is grown with significant amounts of chemical fertilizers that create environmental problems such as soil and ground water pollution. Yet the use of the organic amendment may be less costly and beneficial for maximizing crop yield. Azolla, a small aquatic fern used in rice cultivation in Asia, was tested in this study to improve the productivity of the cabbage (Brassica oleracea) by its use as fertilizer and compare its effects to those of compost and control. The test was conducted according to a device composed of a complete randomized block with 2 repetitions per treatment comprising 4 treatments. The establishment density of cabbage plants is at spacings of 40 cm x 40 cm at the rate of 27 plants per plank on an area of 4 m² per repetition. The treatments were compared, and evaluated by accounting the agronomic parameters: The results showed a greater growth and development of plants on plots amended with Azolla followed by compost compared to unamended plots. The highest yield has been obtained with *Azolla caroliniana* that produced 20.87 t / ha of cabbage. The study showed that *Azolla filiculoides* and *Azolla caroliniana,* under the conditions of this test had great potential for improving the availability of soil nutrients and could provide the needed nutrients amount for cultivation of cabbage without the use of mineral fertilizers.

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INTRODUCTION

Vegetable crops, in particular Brassica genus Brassicaceae, are the most widely used food resources worldwide (Gruarin, 1998). Cabbage is the most widely consumed vegetable in Asia, Africa and America (Talekar*et al*., 1993). The agricultural sector plays a major role in the socio-economic life of Côte d'Ivoire. Vegetable production not only plays a significant role in the country's agriculture and economy, but also meets urban food preferences and demand (Singbo et al., 2004). It is the main source of vitamins and trace elements for the population (Sangaré et al., 2009). Cabbage is an important vegetable in the human diet. It is a valuable source of vitamins A, C and E (Depezay, 2006). It is also a source of mineral salts, mainly iron and cellulose. It has curative, digestive and nutritive properties (Depezay, 2006). It provides the minerals and vitamins needed in a diet and generates substantial income for producers and others involved in the marketing system, reducing poverty and creating jobs. In Côte d'Ivoire, demand for cabbage is growing, and prices are attractive except during periods of overproduction (October-November). The unit price of cabbage can vary from 100 to 400 FCFA/Kg (Dao *et al*., 2003).To meet the high demand for vegetables, growers make massive and abusive use of various types of pesticides and synthetic fertilizers to increase yields. The aim is to ensure the availability of market garden produce for local markets and for export in the event of a surplus. However, the use of these chemicals pollutes the air, upsets soil balance and contaminates groundwater (Cissé, 2000). According to the World Health Organization (WHO), more than a million people are victims of poisoning, twenty thousand of whom have died. All these drawbacks are prompting people to turn to other types of farming that are more respectful of the environment and human health. Organic farming, which favours the use of biofertilizers that contribute to soil enrichment by improving the availability of nutrients to crops, and which leave no toxic residues on produce, is one alternative. Agro-ecological methods are therefore essential to assess cabbage production using an aquatic fern of the Azolla genus containing a species of *Azollae anabaena* algae capable of producing plant-available nitrogen. The general objective of this work is to evaluate the interest of two forage species:

Azolla filiculoides, Azolla caroliniana in relation to compost on cabbage productivity in Daloa. To achieve this general objective. Demonstrate the ability of biofertilizers to fertilize the soil. Increase cabbage yield.

MATERIAL AND METHODS

Fertilizing Materials: The material used as fertilizer includes: *Azolla filiculoides*, *Azolla caroliniana* and compost.

Plant material: Cabbage (*Brassica oleracea* L.): The MAJESTY F1 variety produces apples weighing 1 kg to 1.5 kg in 60 to 65 days after planting in the cool dry season. The apples are very firm and round, and the leaves are crisp and sweet. The variety can be kept in the field for an average of 25 to 30 days after ripening, allowing you to wait until the market is favorable.

Technical equipment: We used the following technical equipment : hoes for weeding and making planks; machetes and rakes, for clearing and cleaning the plot, dabas for ploughing and weeding the plot, watering cans for irrigation, a sprayer for treating the plants , pairs of scissors to remove dead leaves from plants, harvest baskets, a decameter for making boards, stakes used to delimit the beds; each bed is labelled for quick identification of treatments, Finally, mulch and a mosquito net to protect the nursery from wind, defoliating caterpillars, whiteflies, rodents and locusts, A ruler was used to measure leaf length and width, as well as plant spread, HONEST Roberval scale for weighing apples, a digital camera for taking photographs, data collection sheets used to record the data collected.

Compost preparation: Compost preparation required the use of 100 Kg of chicken droppings, 50 Kg of white sawdust, 180 L of tap water, 35 Kg of coffee pulp and 100 Kg of charcoal. Half of each was successively spread out on a black tarpaulin and mixed. The other half was then added to obtain a homogeneous mixture, followed by water (180 L) gradually to obtain a homogeneous heap. Finally, the heap was covered and left to rest for 2 months to allow the compost to decompose and become usable. Compost maturation requires weekly homogenization.

Establishing *Azolla filiculoides* and *Azolla caroliniana* cultivation

Pit preparation: Pits measuring 3 m long, 1 m wide and 30 cm deep were dug using a pick and shovel. Blue plastic bags were placed inside the pits to prevent water leakage.

Liquid manure preparation: The liquid manure was prepared by dissolving 0.5 kg of chicken droppings (*Galusgalus*) in 5 l of tap water. The whole mixture (droppings and water) was left for 2 days in the shade to facilitate decantation by filtration in order to collect the liquid.

Growing Azolla filiculoïdes and Azolla caroliniana: Azolla cultivation involved pouring 125 liters of tap water into a pit, then adding 6 liters of liquid droppings and 125 g of Azolla (Fig 2). Production weights were collected after 3 weeks. Preparation of liquid *Azolla filiculoïdes* and liquid *Azolla caroliniana*. The liquid of the two Azolla species was prepared as follows:In a 15 L bucket, 500 g of Azolla was collected and then mixed to obtain a liquid. This liquid sample was then poured into a 25 L canister.

Cultivation techniques: Before planting, the soil is worked to ensure good aeration and water circulation, and to guarantee good crop rooting.

Nursery installation: The nursery was set up on a 2 m² area marked out with a decameter, rope and stakes. Seedlings were broadcast on December 16, 2017, and the quantity of cabbage seed used was 1 g. Chicken droppings (1 Kg) were applied to the soil. Mulch and mosquito netting were used to cover and protect the nursery from wind, defoliator caterpillars, white flies, rodents and locusts. After sowing, the nursery was regularly watered twice a day, morning and evening. 30 days after sowing in the nursery, the seedlings were transplanted to beds in the field.

Plot preparation: The plot was prepared manually. It was cleared with a machete, then ploughed to a depth of 30-35 cm using a hoe. 8 beds were made using a pickaxe and hoe 2 weeks after the nursery was set up. The dimensions of the beds were as follows: 4 m long, 1 m wide and 30 cm high. The distance between beds was 1 m.

Transplanting cabbage plants: The beds are hoeed before transplanting to loosen the soil. Transplanting involves transplanting the plants from the nursery onto the ploughed surface. To make it easier to pull out the plants, the nursery was abundantly watered just beforehand. The most vigorous plants were transplanted. Transplanting was done on 1 m-wide boards, in triple rows in holes made with the daba. The plants were buried at the limit of the first true leaves, respecting the spacing of 40 cm on the line and 40 cm between the lines, giving 27 plants per bed.

Fertilization of plots: Fertilization was applied in five fractions for all fertilizers.

Azolla :*Azolla filiculoïdes* and *Azolla caroliniana* were applied in liquid form, except for the first application, which was applied in solid form. 1st application: 10 kg of mixed Azolla was incorporated into the soil one week before transplanting. Fresh Azolla after harvesting was kept in a black plastic bag for 6 days in the shade for the first fertilization.2nd application (one week after transplanting): 0.81 L of Azolla (*Azolla filiculoïdes* and *Azolla caroliniana*) was incorporated per plot, i.e. approximately 30 mL of Azolla per cabbage plant.3rd application (two weeks after transplanting) 0.81 L of Azolla (*Azolla filiculoides* and *Azolla caroliniana*) was incorporated per plot, i.e. approximately 30 mL of Azolla per cabbage plant. 4th application (three weeks after transplanting) 0.81 L of Azolla (*Azolla filiculoïdes* and *Azolla caroliniana*) was incorporated per plot, i.e. approximately 30 mL of Azolla per cabbage plant. 5th application (four weeks after transplanting) 0.81 L of Azolla (*Azolla filiculoïdes* and *Azolla caroliniana*) was incorporated per plot, i.e. approximately 30 mL of Azolla per cabbage plant.

Compost: Compost was incorporated at a rate of 34.3 Kg per plot:1st application: incorporated one week before transplanting, i.e. 10 Kg of compost in the soil of each plot.2nd application: one week after transplanting: 150 g of compost per cabbage plant, i.e. 4.05 kg of compost for each plot.3rd application: two weeks after transplanting: 150 g of compost per cabbage plant, i.e. 4.05 kg of compost for each plot.4th application: three weeks after transplanting: 300 g of compost per cabbage plant, i.e. 8.1 kg of compost for each plot.5th application: four weeks after transplanting: 300 g of compost per cabbage plant, i.e. 8.1 kg of compost for each plot.

temoin: The control plots received no fertilization**.**

Experimental set-up: The experimental design is a complete randomized block with 2 replicates of 4 treatments each. The block is subdivided into elementary plots of 4 m × 1 m each, i.e. 4 m2 in area. The spacing between elementary plots was 1m. The trial was conducted over a total area of 72 m^2. Cabbage plants were planted at 40 cm x 40 cm spacing, with 27 plants per bed and 4 m² per replicate (trial), i.e. 8 m² per treatment (Fig 2).

Data collection: The following data were collected: recovery rate, leaf length and width, number of leaves, plant spread and yield. Measurements were taken on 10 fixed and numbered plants per bed, i.e. 20 plants per treatment. These plants were selected at random. Measurements were taken once a week from 44 days after sowing to 65 days after sowing, i.e. 4 times during the cycle corresponding to times T_1, T_2, T_3 and T_4.

Rework rate: The recovery rate (T) was calculated at the start of vegetation as the ratio of the number of plants surviving one week after transplanting.

T= (number of plants transplanted) /(number of plants surviving)×100 **(1)**

Leaf length and width: Leaf length was measured using a tape measure between the tip of the leaf to be measured and the point where the petiole is attached to the stem, and leaf width was measured using a tape measure between the tips of the leaf and the median of the leaf.

Plant span: Plant span was also measured using a tape measure between the tips of the terminal leaflets of the 2 outermost leaves of the cabbage plant.

Yields: Yields (T ha^{-1}) were assessed at 84 days after transplanting, using the following formula:R=M/S **(2)**

With: R = yield in T per ha;
M = total weight of apples harvested, in tons ;
S = surface area, in ha

Data analysis: Data were encoded using Excel spreadsheets. The various analyses were carried out using STATISTCA 7.1 software, and data processing was based on the analysis of variance and multiple comparison of means test. Shapiro-Wilk normality tests were applied to all variables prior to all analyses. This ANOVA test showed the significance of treatment effects on growth and yield parameters. In this condition, Tukey's test was used to classify two-by-two means when variables showed a difference ($p < 0.05$) between treatments. Differences are significant for a probability value of less than 0.05.

RESULTS

Variations in growth and yield parameters

Recovery rate: The recovery rate obtained with the different fertilizations was maximum (100%) for all treatments, including the control (fig 3).

Cabbage leaf length and width: Analysis of variance of cabbage leaf length reveals that the effect of fertilization on leaf length is significant at the 5% threshold from the first week for all treatments. Leaf length was greater with *A. filiculoïdes* (28.27 cm and 31.94 cm) and *A. caroliniana* (25.43 cm and 30.18 cm) than with compost (24.08 cm and 25.28 cm)

A: Pit covered with blue bag; B: Pit filled with water; C: Liquid manure in pit; D: Inoculation of Azolla in pit

Fig. 1. Setting up *Azolla cultivation*

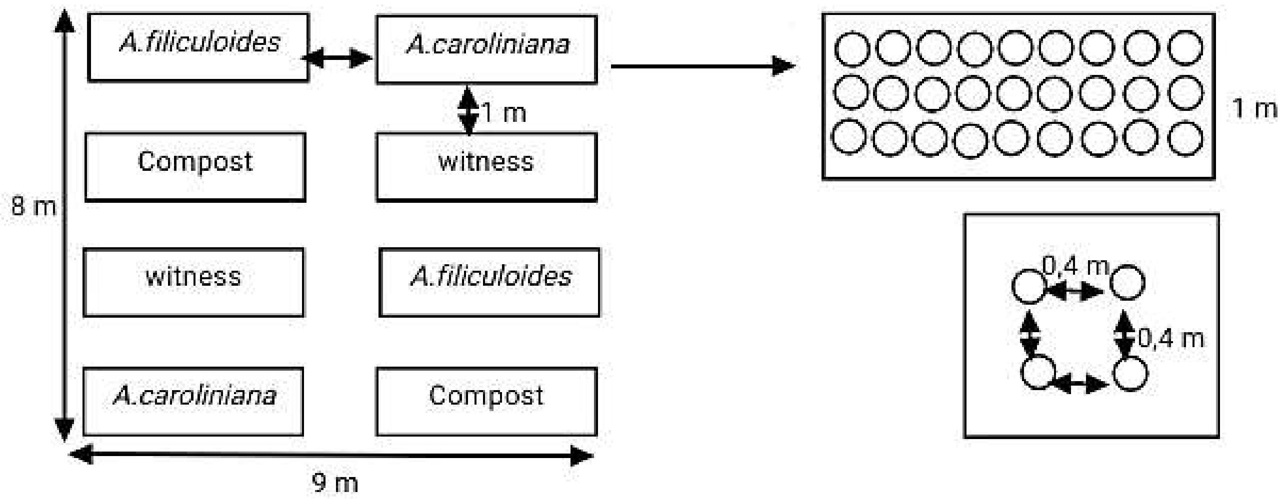

Fig 2. Experimental set-up

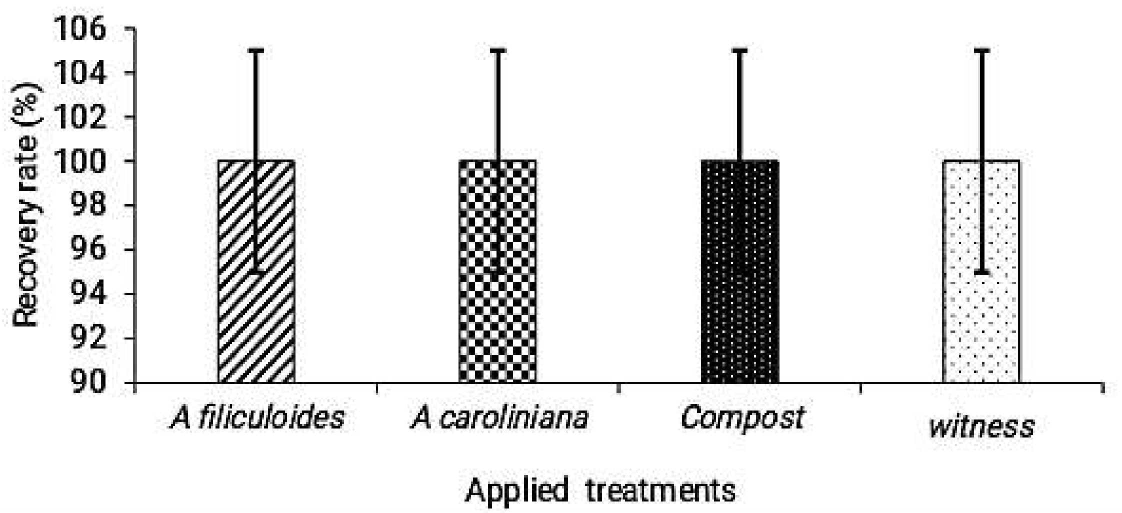

Fig. 3. Recovery rate of cabbage 7 days after transplanting

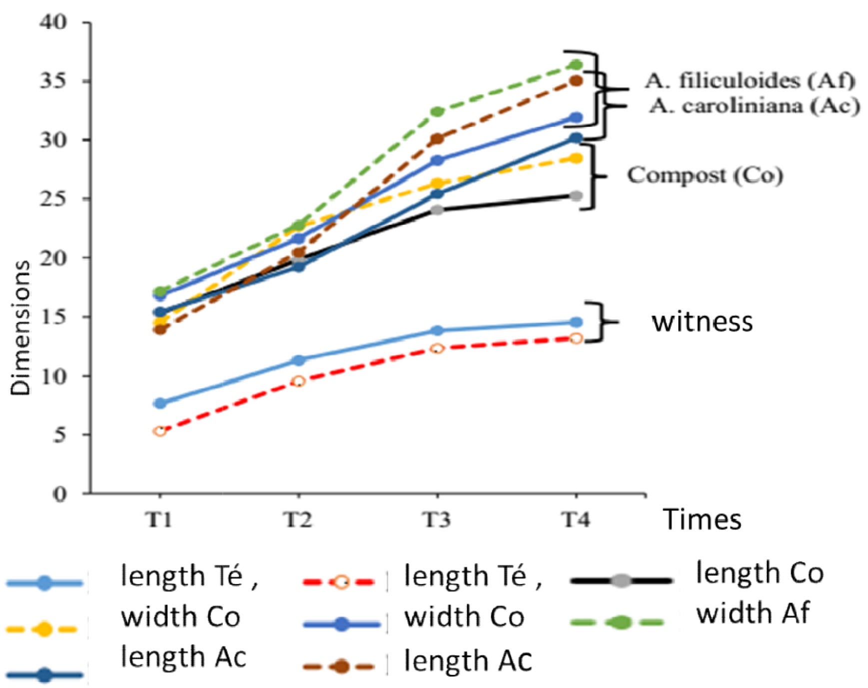

Fig 4. Evolution of cabbage leaf length and width

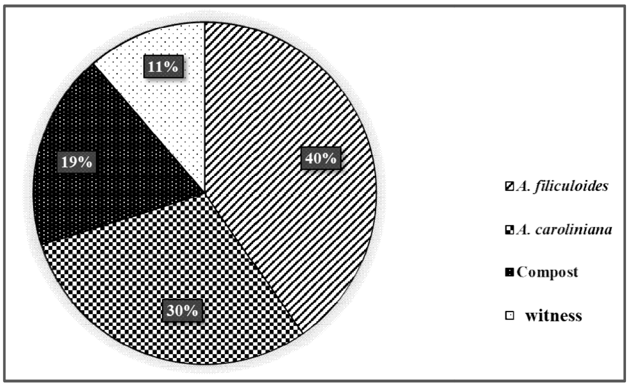

Fig. 5. Diagram of number of apples per fertilization

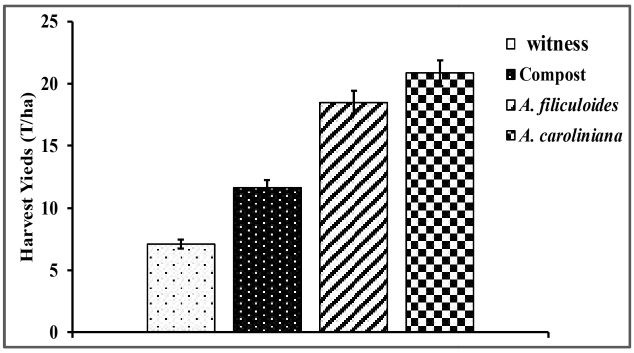

Fig. 6. Yield evolution diagram

and the control (13.84 cm and 14.56 cm) at times T_3 and T_4 (fig 4). The effect of fertilization on cabbage leaf length in the different treatments shows that the *A. filiculoïdes* treatment gives the greatest length. Treatment of cabbage with compost reveals that growth in plant leaf length becomes progressively weaker (from 24.08 cm to 25.28 cm) from T_3 to T_4, whereas it continues to increase under fertilization with *A. filiculoïdes* (from 28.27 cm to 31.94 cm) and *A. caroliniana* (from 25.43 cm to 30.18 cm) at constant doses over time. However, this growth becomes weak from the time of apple set.Analysis of variance showed that fertilization had a significant effect at the 5% threshold on cabbage leaf width from the first week for all treatments. Treatments with *Azolla filiculoïdes, Azolla caroliniana* and compost resulted in better leaf width growth, which was significantly different from that induced by the control. With these three treatments, leaf widths measured 32.41 cm for *Azolla filiculoïdes*, 30.12 cm for *Azolla caroliniana* and 26.33 cm for compost at T_3, whereas at the same date, these widths with the control were 12.35 cm. At T_4, the average width of cabbage leaves treated with both Azolla species is wider (36.4 cm and 35.06 cm) and significantly different from that induced by compost (28.47 cm) and the control (13.2 cm). This difference is clearly seen in figure 8, which shows the evolution of leaf width over time (fig 4).

Number of leaves on cabbage plants: Analysis of variance of the number of cabbage leaves reveals a significant effect at the 5% threshold from the first week. The number of leaves emitted by the plants was higher when fertilized with the three biofertilizers (Table I). 4 weeks after transplanting, the effect of fertilization on the number of leaves emitted by cabbage plants in the different treatments shows that *A. filiculoïdes* (21.7 cm) induces significantly higher leaf emission than the other organic treatments (19.9 cm for *A. caroliniana* and 20.10 cm for compost) and without fertilizers (11.50 cm). Biofertilizers result in greater foliation than foliation without fertilizers. In all treatments, this leaf formation stops at bud-break.

Size of cabbage plants: Analysis of variance of soil coverage by cabbage plants reveals a significant effect at the 5% threshold from the first week for all treatments. The wingspans of plants fertilized with both Azolla species and with compost were roughly equal but greater than those of cabbage plants without fertilization (Control) at T1 and T2 (Table II). The compost treatment reveals that the increase in plant span decreases with increasing compost dose, becoming smaller at 54.27 cm at T4, even though this amount of fertilizer is increasing. On the other hand, cabbage plants fertilized with *A. filiculoides* and *A. caroliniana* show greater growth in wingspan at T4, reaching 61.94 cm and 67.35 cm respectively. The increase in wingspan ceases with apple set.

Crop yields

Number of apples at harvest: Treatments with *Azolla filiculoïdes* and *Azolla caroliniana* yielded high proportions (40% and 30%), ahead of compost (19%) and the control (11%) (Fig 5).

Harvest yields: The Azolla treatments produced higher yields, which differed from those of the compost and control treatments. Plants fertilized with *Azolla caroliniana* and *Azolla filiculoïdes* had high yields of 20.87 T/ha and 18.51 T/ha respectively, followed by the compost treatments 11.63 T/ha, and the control 7 T/ha (fig 6).

DISCUSSION

The average leaf length of plants fertilized with the two Azolla species was greater and significantly different from that induced by compost and the control at the third and fourth week after transplanting. No significant difference was observed in the evolution of leaf number between plants fertilized with *A. filiculoïdes, A.caroliniana* and compost. However, their action was different from that of the control. In sum, the number of leaves emitted by cabbage plants fertilized with *A. filiculoïdes* was significantly higher than in the other organic treatments. Treatments with *Azolla filiculoïdes, Azolla caroliniana* and compost resulted in better growth in leaf width, with a significant difference compared with the control. Plants grown with *Azolla filiculoïdes* and *Azolla caroliniana* had wider leaves. The wingspan of plants fertilized with *Azolla filiculoïdes* and compost was roughly equal to and greater than the wingspan of cabbage plants fertilized with *Azolla caroliniana* during the first two weeks. From the third to the fourth (Fig 6). Cabbage yields varied from one treatment to another.week, the average wingspan of cabbage plants treated with both Azolla species is larger and significantly different from that induced by compost and the control. *Azolla filiculoïdes, Azolla caroliniana* and compost and clearly had an effect on vegetative parameters.From this study, it emerges that the growth of cabbage plants fertilized Azolla is higher and significantly different from the growth of cabbage plants fertilized with compost and without fertilizer. In fact, these results are in line with those of Ouattara (2018), who recorded increases in tomato stem height, collar diameter and leaf length compared with compost and the control after the application of *Azolla caroliniana*.The recovery rate was maximum for all treatments, showing that organic fertilizer applications had no effect on this parameter. This may be explained by the selection of vigorous plants prior to transplanting. Indeed, plant vigor influences recovery. Numerous studies, including those by Bhardwaj et al, (2000), Olaniyi et al, (2010), Ojetayo et al, (2011) and Musas (2012) have shown that the recovery rate of cabbage, Chinese cabbage, tomato, spinach and onion was similar on unfertilized soil, NPK 15-15-15 and various manures.According to Dobermann et al (2000), nitrogen fertilization affects all the parameters contributing to good yield. The Azolla treatment gave the best growth. *A. filiculoides* and *A. caroliniana* ensure good cabbage growth. This is made possible by the permanent availability of assimilable nitrogen to the cabbage plant by Azolla, as shown in the work of Maria Andrea et al. (2007), and could be linked to significant mineralization of soil nutrients in the beds treated with Azolla. Thanks to the heterocysts contained in Azolla cells, which are atmospheric nitrogen-fixing sites (Dupuy et al., 1987), the fern fixes and accumulates nitrogen, which it transforms into ammonia.

This form of assimilable nitrogen, along with other minerals, is released progressively, ensuring its availability when the plant actually needs it, and enabling it to grow. In terms of the number of leaves and spread of cabbage plants treated with Azolla, the superiority is also linked to the large quantity of nutrients made available to the plant. Organic matter (Azolla) contains a significant quantity of elements such as nitrogen and phosphorus, which are essential for plant growth and development and have an immediate effect on foliage development and crop production (Brasset& Couturier, 2005).The results indicate that the compost applied did not result in higher vegetative growth than Azolla, probably due to the low availability of their nutrients, particularly nitrogen. The difference in vegetative growth between cabbage treated with compost and cabbage treated with Azolla can be explained by the fact that the nitrogen in the compost was mainly in organic form, and its mineralization during the vegetative period was probably not sufficient to meet the nitrogen requirements of the cabbage plants. The effect of compost through organic matter on vegetative growth, demonstrated by the results obtained in this trial, are confirmed by other authors such as N'Dayegamiye *et al*., (2004) who recorded significant increases in barley growth parameters and yields, compared with the control in line with the results of Toundou, (2016) on maize growth parameters compared with plants grown on the control soil without amendment explained by the ionic balance of nutrients in the soil amended with this compost. Indeed, according to Toundou (2016), there is an ionic balance necessary for the absorption of the nutrients the plant needs to grow well. Thus, the balanced ion content of this compost enabled it to improve plant growth.

The superiority of compost over the control would then be linked to the combined action of improved soil properties and nutrient mineralization.Indeed, Mulaji (2011) showed that the rate of organic matter decomposition and plant growth was closely linked to the timing between nutrient release and plant uptake. The control with no fertilization had the lowest growth. The poor performance of cabbage plants observed in the control plots can be attributed to the physico-chemical conditions of the soil (Mukalay*et al*., 2008; Kasongo, 2008). Fertilization is therefore correlated with the vegetative parameters of the plants, which is in line with the results of Kimuni et al. (2014), who reported that growth and yield of Chinese cabbage were considerably improved following the application of different doses of composts and NPK mineral fertilizers. Finally, these studies show that good cabbage growth is one of the sine qua non conditions for very good cabbage yield.The results show that under organic fertilization of cabbage with *Azolla caroliniana* and *Azolla filiculoides*, the number of apples obtained is considerable. Cabbage apple yield increased by 13.81 t/ha or 24% for Azolla caroliniana, and 11.45 t/ha or 20% for Azolla filiculoides, compared with the unfertilized control. This is in line with the results of Diara (2000), who showed that the use of A. pinnata increased rice grain yield in Senegal. These results are also in line with those of Lumpkin-Plucknet (1982), who conclude that *A. pinnata* is a green manure that significantly increases rice yield. Nitrogen is the main factor in crop growth and yield among the main mineral elements in plants (FAO, 1980). This increase in yield is due to the permanent availability of assimilable nitrogen to the rice plant, as shown by the work of Maria Andrea et al. (2007). According to Razaf*et al*, (1988), the microscopic characteristics of the *Azolla pinnata* symbiosis lie in the presence of numerous sites of fixation and transformation of atmospheric nitrogen into permanently assimilable nitrogen.

This work is also in line with that of Rakotonasolo (1988), who asserts that *A. pinnata*significantly improves rice yields in Madagascar. This is also confirmed by Reynaud (1984), who asserts that, in the dry tropical zone, the use of *A. pinnata* in rice cultivation is an important agronomic prospect.This study also showed that, under organic compost fertilization, the number of apples obtained was high and cabbage apple yield increased by 8%, or 4.57 t/ha, compared with the control. The positive role of organic matter through compost on yield, demonstrated by the results obtained in this trial, are confirmed by other authors such as N'Dayegamiye*et al*, (2004) who recorded significant increases in barley yields, compared with the control, after application of mixed sludge and manure. This degree of superiority in cabbage yields under organic compost fertilization compared with the control further demonstrates the importance of compost use and also this superiority is due to the incorporation of hen droppings, known to be rich in phosphorus during composting. Phosphorus is an important element in fruit production (FAO, 2000). This corroborates the results of Useni*et al*, (2014) on the cultivation of Chinese cabbage after application of hen manure composts. According to Charland et al, (2001), good compost increases yields compared with unfertilized soils, even when applied at low doses.

CONCLUSION

The results obtained illustrate the beneficial effects of Azolla produced on cabbage cultivation, both on the morphology of the plant.The analysis of the results relating to the different treatments applied on the parameters studied showed that Azolla recorded appreciable increases compared to compost and the control. Thus, these treatments applied influenced in a very highly significant way on the growth parameters studied.The best results were recorded at the level of *Azolla filiculoides* and *Azolla caroliniana*.The permanence of assimilable nitrogen around the plants and the improvement of the physicochemical and biological properties of the soil influenced the supply of nutrients to the plants.

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