

Effect of Culture Density of Black Nightshade (*Solanum nigrum*) on the Insect Infestation

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Abstract: Leafy vegetables to which the black nightshade belongs occupy an important place in the world economy and contribute effectively to food security. But their culture is limited by the aggressiveness of pests and diseases. The study was based on a trial in the field in the experimental plots of IRAD-Ekona. Four density levels (T1, T2, T3 and T4) respectively corresponding to distances: random, 20cm x 20cm, 30cm x 30cm, 40cm x 25cm were tested. The results obtained and presented here show that in general, *S. nigrum* is strongly attacked by three types of insects: aphids, black ants and whiteflies. The citation order marks the order of importance of the attack. Indeed, when the culture densities are large (20cm x 20cm or random), *S. nigrum* is subject to many attacks. Culture density positively influences certain growth parameters ($p \leq 5\%$) and the yield of all varieties studied. The high densities (T1 and T2) are an ideal ecosystem for the attraction and development of insects; major disease vectors. The T3 treatment (30cm x 30cm) could be recommended to farmers to improve cultivation techniques and effectively increase their yield.

Keywords: *Solanum nigrum*, Densities, Insect, Infestation, Yield

1. Introduction

The culture of leafy vegetables occupies an important place in the world economy and contributes effectively to food security. Among these leafy vegetables, black nightshade (*Solanum nigrum* L.) is one of them. The genus *Solanum* is the largest of the *Solanaceae* family. It includes at least 1500 species worldwide [1], with plants such as tomato (*S. lycopersicum*), potato (*S. tuberosum*), eggplant (*S. macrocarpon*) whose importance as a vegetable and economic value are no longer shown. The family is widespread in tropical and temperate regions of the world with great concentration in Central America, South and Australia [2]. *S. nigrum* morphogenically includes many different taxa in all parts of the world. The tropics of the

America are found to have the largest number of species, but not *S. nigrum*, which appears to be a species of Eurasian origin [3]. Yet in the former regional floras, all taxa belonging to the *Solanum* section are identified as *S. nigrum* so that references to this species in botany and ethnobotany literature should be considered with great caution [4]. Edmonds et al., [1] estimates that over 300 specific names and specific intra published since the six varieties described by Linne under the binomial *S. nigrum* in 1753, so that we find many synonyms. Furthermore, the boundaries between many species are poorly defined, with many "new" taxa that are proving to be anything but tiny versions of those already described.

Black nightshade with broad leaf is grown widely in temperate regions, tropical rain [5, 2]. In Africa, mainly in

Ivory Coast and Cameroon, it is grown on small farms and vegetable gardens on the outskirts of major cities for market supply. However there are no reliable statistics on production and trade. Nevertheless, we note small exports from Cameroon to Nigeria and Gabon. It is a fast growing plant of high nutritional value [6]. Excellent source of iron, calcium, protein, vitamin A, C, iodide, zinc and low in fat consumption, it generally provides few calories to the body [1]. More leafy vegetables contain more soluble fiber than insoluble fiber [7], which facilitates digestion. The leaves and fresh shoots are widely used as a cooked leaf vegetable. They are served with fufu corn, plantains, sweet potatoes, potatoes, yams, corn, or pounded taro. In Cameroon, black nightshade is appreciated as the "Osam" or "zom", in Ivory Coast as the "fouet", Benin "oyomoh", Nigeria "ogunmo" or "Odu" [4]. Apart from being used for human consumption, the leaves are also used as fodder for livestock and wild herbivores [8]. Nightshade is traditionally used as a medicinal plant particularly to treat heartburn. The plant extracts are used as an analgesic, antispasmodic, anti-inflammatory and vasodilating [9]. Similarly, the plant is also used as an insecticide and larvicide [10]. Similarly, pigments (anthocyanins) from the violet and black fruit are used as ballpoint dye or ink. All *Solanum* species contain spirosolane alkaloids, glycoalkaloids, including solanine and solanidine, the latter being less toxic. They have a bitter taste and can be poisonous if eaten frequently [11].

The culture of black nightshade associated or monoculture is a source of income for vulnerable populations [7,12]. The southwestern region Cameroon is very favorable to culture. However, several factors limit its production, including common pests such as locusts (*Zonocerus variegatus*), beetles (*Podagrica spp*); black aphids (*Aphis fabae*), nematodes (*Meloidogyne spp*), flea beetles (*Epilachna hirta*) [13]. Effective control of these pests is achieved in the most productive countries by using insecticide sprays [14]. Many aphids are capable of infesting the underside of the leaves and

cause them winding, and the tops of young infested plants can grow more [14]. All these pests are vectors of viral diseases, such is the case of virus yellows ribs (observed in Cameroon and Nigeria) probably transmitted by *Bemisia tabaci* [15]; fungal diseases such as late blight (*Phytophthora infesting*) that cause seedling mortality in the nursery; the early blight (*Alternaria solani*) causing the appearance of rounded spots on leaves; leaf spot and leaf mold having respectively pathogen *Cercospora celosia* and *Cladosporium oxysporum* characterized by the appearance of gray spots on leaves. A bacterial wilt (*Ralstonia solanacearum*) was found with attack by 1% in the rolling farmland of West Java [16].

Culture density significantly influence crop yield. However, distances of 15 to 50cm between plants left encourage a good leaf production [17]. For better production of seed, spacing of 75 to 120cm between the plants should be used [14]. These various data presented lead us to understand clearly that culture density can acceptably influence the growth and yield of crops. Is this influence due to one factor of competitiveness of the plants on their acreage? Don't insect pests and disease vectors have a preference in relation to the density of cultures? In other words, does the spatial structure of the host population influences the development of diseases?

The aim of our study is to contribute at increasing the productivity of the black nightshade by reducing losses and damage caused by pests. More precisely; identify insect pests that infest *S. nigrum*, study the effect of culture density on the infestation of insects and their impact on performance.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The seed used was three improved varieties of nightshade: BG24 native of Tanzania, SS52 from Cameroon and MW25 from Kenya (Table 1).

Table 1. Phenotypic description of varieties of seed used.

Varieties Elements	BG24	SS52	MW25
Originally	Tanzania	Cameroon	Kenya
Location 1st inflorescence	along the main stem	along the main stem	on a main branch
Inflorescence type	Umbel	Umbel	Umbel
Peduncle	Horizontal	Horizontal	Vertical
Color of the mature berry	dark purplish black	dark purplish black	dark purplish black
Attachment of the fruit on the stem	The fruits remain on mature plants	The fruits remain on mature plants	The fruits remain on mature plants
Color of the chair of fruit	Purple	Purple	Dark purple
Color of the stem and branches	Green	Light purple	Dark purple
Leaf margin	Smooth	corrugated	Smooth
Leaf shape	Round	Oval	Oval
Summit leaves	Obtuse	Intermediate	Acute
Leaf color	Green	Dark Green	Dark Green
Hairiness of the leaves	pubescent leaves	Glabrous	pubescent leaves
Branching out	primary and secondary branches	primary and secondary branches	primary and secondary branches
Hair rods	Smooth	Smooth	Pubescent

2.1.2. Presentation of the Study Area

The Institute of Research for Development (IRAD) is

located at the entrance of the village Ekona in the southwestern region of Cameroon with an altitude of 400 m and covers an area of 55km². The IRAD Station is at 06 ° 30

'00 latitude and 08 ° 30' east longitude 00.

Climate IRAD-Ekona is equatorial (Cameroonian type). The average temperature is 24.4°C during the dry season and the rainy season 23.7°C. It's a two-season climate: a long rainy season from March to October and very severe during the months of July, August and September followed by a short dry season from 4 November to February. The average annual rainfall is about 2000mm.

The soil is dark volcanic type containing a mixture of clay, sand, rock and gravel. Their fertility is very high and climatic factors are favorable for agricultural development, which has also attracted agricultural companies such as CDC.

2.2. Methods

2.2.1. Nursery

An area of 2m² was built and improved near the work site, with black earth mixed with chickens' dung. The seed beds were pretreated with insecticide (moucap). All three seedbeds corresponded to a specific variety of *S. nigrum*. After sowing, the seed beds were covered with a thin layer of earth and very light green leaves, with the aim of preventing the ants and the wind from carrying the seeds from one bed to another, and also protect the beds during heavy rains. Thereafter, watering is done once every two days, in the absence of rains. The first exercise was observed 4 days after sowing (DAS). After the first exercise, the green leaves that covered the beds were removed and the nursery was protected by a mosquito grid (Figure 1), this in order to avoid the influence of rainfall. From that date, the germination rate was assessed by counting the germinated seeds in comparison with seeds placed in nurseries.



Figure 1. Nursery aged 26 DAS.

2.2.2. Experimental Set-Up

A plot of 122.5m² including 36 remote elementary plots 0.5m allocated among the three varieties was laid on September 8, 2014. Each measuring 2m long and 1m wide and included a definite number of plants according different densities cultures or treatments (Figure 2):

- For T1, the plants were randomly arranged and contained 50 plants
- For T2, the plants were separated from 20cm by 20cm and contained 50 plants
- For T3, the plants were separated from 30cm by 30cm and contained 21 plants
- T4, the plants were separated from 40cm by 25cm and contained 20 plants

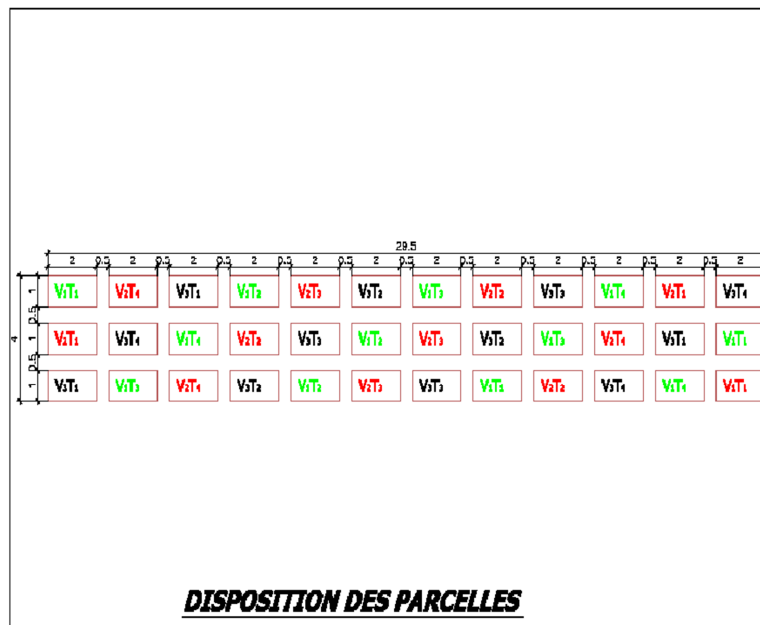


Figure 2. Experimental design.

V1- BG24 native of Tanzania
V2- SS52 from Cameroon
V3- MW25 from Kenya.

2.2.3. Transplanting Field

Two days before the transplantation more precisely October 4, 2014, elementary plots were returned and mixed with the ashes of palm nuts, the dose of 06 kg per parcel. Four weeks after sowing or 26 DAS (Figure 1), the plants about 6 to 8 cm in height were close for transplanting in the field. Before transplanting, the blocks were sufficiently watered to facilitate the adaptation of young plants in their new environment.

2.2.4. Parameters Evaluated

The growth parameters were evaluated as follows:

- the germination rate (T) was determined by the formula:

$$T = \frac{\text{Number of germinated seed}}{\text{Number of seed sown}} \times 100 \quad (1)$$

- Ten plants were chosen weekly randomly in each plot for evaluating the diameter of the collar, the number of leaves, the length of the stem and leaf area, this from the 14th day after transplanting (DAT). The evaluation of these parameters has stopped a week before the first harvest.

$$\text{Area (m}^2\text{)} = \text{length of blade} \times \text{blade width} \times \text{leaf count} \times 0.5296 \quad (2)$$

- Performance was evaluated after the first harvest.

$$\text{Yield (t.ha}^{-1}\text{)} = (\text{Mass vegetables}) / (2\text{m}^2 \times 3) \times 10000 \quad (3)$$

2.2.5. Pathological and Entomological Settings

The number of leaves infested plants was evaluated from the 14th day after transplanting.

Ten plants randomly chosen in each plot used to the weekly evaluation of the number of aphids, snails, caterpillars, ants and whitefly present on 5 leaves, this from 18th DAT (Figure 3). The evaluation of these parameters was stopped two weeks before the first harvest.



Figure 3. View of some plots 18th DAT.

2.2.6. Data Analysis

Data was analyzed using the computer program, software analysis system (SAS), which uses the analysis of variance (ANOVA) and the test of Student-Newman-Keuls at 5% to compare the means. Whenever there was a significant difference between the means, the least significant difference (LSD) method was used to separate them.

3. Results and Discussion

3.1. Germination Rate

The first sprouts appeared the 4th DAS and stopped the 8th for SS52 and BG24 variety and finally the 10th DAS for MW25 variety (Fig. 4). The germination rate reached its maximum value at the 10th DAS, then it is 98.8%, 99.8% and 95% respectively for the BG24, SS52 and MW25 varieties. The ideal is naturally a germination rate of 100 % for all varieties. Indeed, these results are not far from those obtained by Fontem and Schippers [18] which emphasize in this regard, that the germination of seeds can sometimes be a problem because of a reduced force due to poor seed extraction and therefore an improper disposal of sugars and germination inhibitors present in fruits. This germination problem can also be due to the fact that the seeds were not well dried and stored, or are dormant.

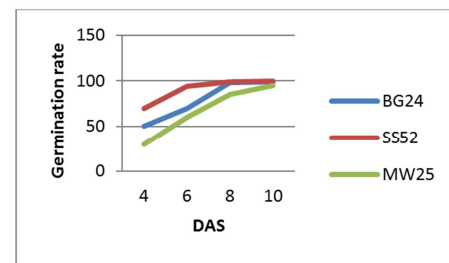


Figure 4. Evolution of the germination function of time.

3.2. Number of Insects Per Plant

3.2.1. Number of Aphids Per Plant

The results obtained by us in this work show that in general, when the black nightshade varieties are arranged T4 and T3 treatment, they are less attacked by aphids (10 aphids average) while they are attacked by these aphids when subjected T1 or T2 treatment (an average of 13 aphids per plant arranged according to the treatments T1 and T2).

Figure 5 watches varieties BG24 and MW25 suffer less attack of aphids when subjected to the T4 treatment (on average 9 aphids) compared to the range MW25 when subjected to treatment T1 (14 on average aphids). The comparison of means test shows a significant difference between the two averages ($P \leq 5\%$).

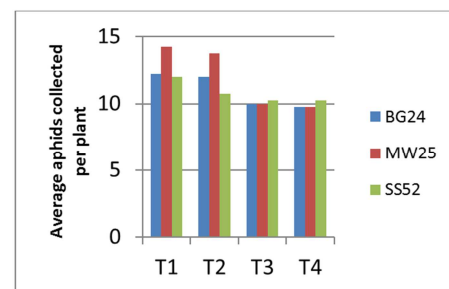


Figure 5. Evolution of aphids per plant under the effect of treatments and varieties.

3.2.2. Number of Ants Per Plant



Figure 6. Presence of black ants on the leaves of the black nightshade.

In general, black ants are attacking more black nightshade plants arranged according to the experimental protocol of the T1 or T2 treatment. Indeed, on average, 3 ants are recorded on a black nightshade plant, while two ants are observed on those arranged in the T3 or T4 protocol (Figure 6).

Regarding varieties, BG24 variety is less attacked by the black ants. Indeed, on a plant of this variety, there are on average two black ants when subjected to T3 treatment. The information here is striking that the most attacked treatment is the type T1 to an average of 5 ants per plant in the variety SS52 (Figure 7).

The point to make here is that the difference in the number of attacks recorded between the treatment of T1 and T3 variety SS52 treatment BG24 variety is highly significant ($P \leq 5\%$).

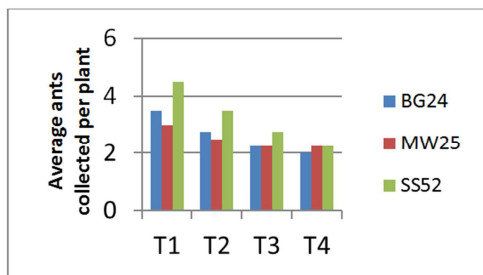


Figure 7. Evolution of ants under the effect of treatments depending on the variety.

3.2.3. Number of White Flies Per Plant

Figure 8 shows that the varieties are attacked by white flies when subjected to treatments T1 and T2 and less when subjected to the T3 and T4. However the average maximum flies was recorded in T3 for MW25 variety (7 flies) and the minimum to the same treatment with varieties BG24 and SS52 (4 flies).

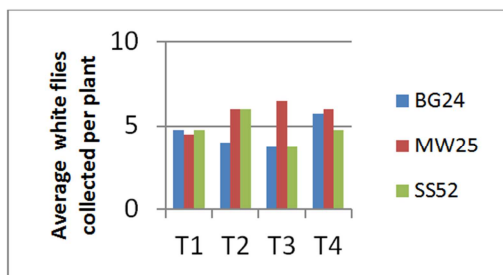


Figure 8. Change whiteflies under the effect of treatments depending on the variety.

3.2.4. Number of Snails Per Plant



Figure 9. Snail perched on a black nightshade plant.

The analysis of average data on the number of snails shows that they are very present on the black nightshade plants. Indeed, when collecting data, we noticed their presence (Figure 9)

3.2.5. Number of Caterpillars Per Plant



Figure 10. Caterpillar on black nightshade plant.

Analysis of data on the average number of caterpillars shows they are less present on the black nightshade plant. However, when collecting data, we noticed their presence (Figure 10).

In general, black nightshade plants are heavily attacked by three types of insects: aphids, black ants and whiteflies. The citation order marks the order of importance of the attack. Similar results were found by Fondio *et al.*, [15]. Aphids mostly attack black nightshade plants with high plant density (T1 and T2) then they are less attacked with low seeding rate (T3 and T4). The high seeding rate proves to be a favorable ecosystem to the development of aphids and ants. Varieties BG24 and MW25 are attacked by aphids, on average, 12 are present on plants of these two varieties. However, the variety SS52 is less attacked; on average, 10 are attracted by the variety.

3.3. Number of Leaves and Plants Infested

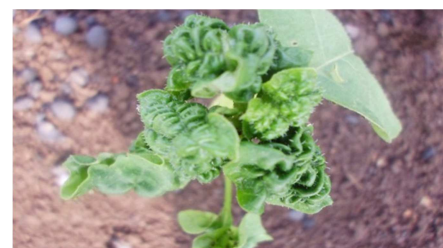


Figure 11. Black nightshade leaves wrapped.

Data analysis (Figure 12) shows that black nightshade is infected when subjected to treatments T1, T2 and less to T3 and T4 treatments. Tukey Multiple comparison's test show a significant difference between the pairs (T1, T2) and (T3, T4) $P \leq 5\%$. It appears from these data that the BG24 variety is more resistant when subjected to T3 treatment (average of infected leaves = 4.41), while the MW25 is the most sensitive in T1 (average = 10.97 infected leaves).

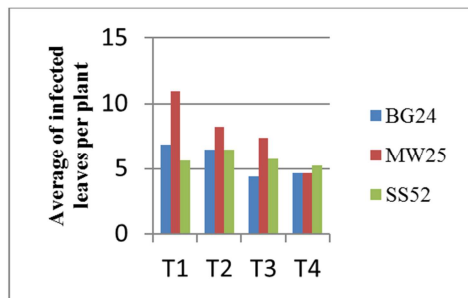


Figure 12. Evolution of the number of infested leaves per plant depending on the varieties under the effect of treatments.

While varieties BG24 and SS52 have no significant differences in the number of infested leaves between treatments, the variety MW25 meanwhile presents very significant differences between treatments ($P \leq 5\%$). It shows that the T4 treatment is that which exposes fewer plants to pathogen attack and variety MW25, the more susceptible to disease. Indeed, the spatial structure of the host population influences the epidemic development speed in the case of local transmission of the disease. This effect has been demonstrated experimentally, for example in the case of withering of the seed of the rice due to *Rhizoctonia solani* [19] and explained from a theoretical point of view, either as part of a transmitted disease by a vector [20] or in the more general context [21]. The aggregation of plants increases the incidence of early epidemic disease and decreases end [22, 23]. Where plants are randomly distributed, the disease spread substantially the same in all directions. Whereas when the plants are distributed online, the dispersion is preferably carried along the line [24]. The most observed symptoms are: windings, wilting and yellowing of leaves (Figure 11). These symptoms are usually observed in the case of diseases caused by viruses and bacteria; however black aphids (*Aphis fabae*), beetles (*Lagria spp.*, *Podagrica spp.* and *Epilachnahirta*) and nematodes are the main vectors of these diseases [18]. It is therefore understandable why the BG24 variety subjected to treatment with high density, where it attracts more insects, is also the most attacked by the disease. MW25 variety is more resistant to diseases while SS52 variety is more resistant to insects (aphids).

3.4. Length of the Rod and Collar Diameter

The analysis of the mean values over the length of the rod and collar diameter shows a variation with time for each treatment with significant differences (p value ≤ 0.050). At the sixth week, the maximum length of the stem was

observed for the variety BG24 (65.83cm) when T3 was subjected to treatments and the minimum length to the variety MW25 (40.25cm) when subjected to treatment T1 (Figure 13). At the same time, the maximum collar diameter was observed in the same variety BG24 (0.88cm) when subjected to the same treatment T3 (Fig. 14). The maximum and minimum heights observed in our study are the same as those found Andes [25]. Following the results, we note that only the T3 treatment (30cm x 30cm) makes a good redistribution of organic substances produced by photosynthesis.

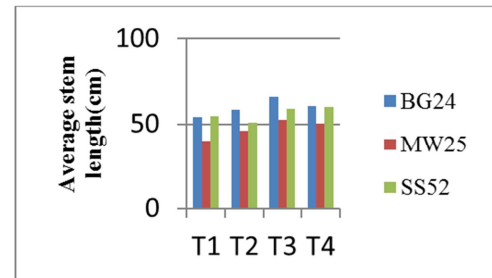


Figure 13. Change in the length of the stem of plants under the effect of treatments.

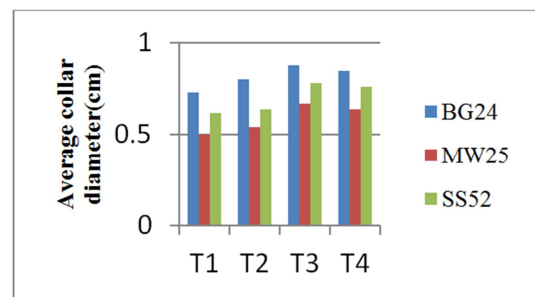


Figure 14. Evolution of the collar diameter of plant under the effect of treatments.

3.5. Number of Leaves Per Plant

The analysis of average data on the number of leaves produced for all varieties showed a significant difference between T1 and T3 treatments; T1 and T4; T2 and T3; T2et T4 (p value $\leq 5\%$). The maximum leaf production was observed when plants were subjected to T3 treatment (56.73) and a minimum value, when subjected to T1 treatment (Figure 15). The abundance of leaves in T3 and T4 treatment plants explains their good growth in diameter and length.

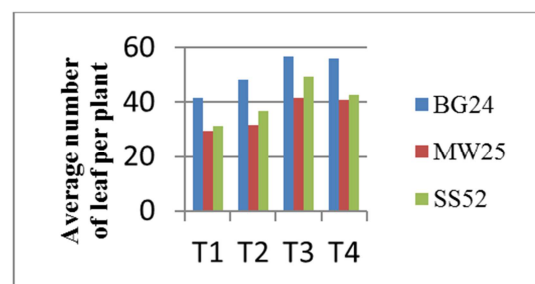


Figure 15. Evolution of the number of plant leaves under the effect of treatments.

3.6. Evolution of Leaf Area

The average leaf area increases from the first to the fifth week and continues to grow in the sixth for all treatments. T1 Treatment records the lowest average for all varieties 27.82, 26.00 and 23.42 for BG24, SS52 and MW25 respectively, while treatment T3 records maximum values 37.83, 37.02 and 30.48 for BG24, SS52 and MW25 respectively (Figure 16). Indeed Turkey multiple comparison's test shows a significant difference between the pairs (T1, T2) and (T3, T4) P-value $\leq 5\%$.

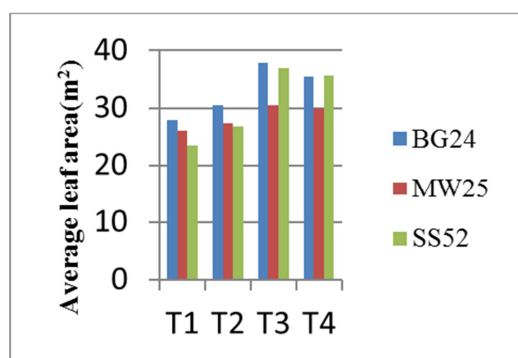


Figure 16. Evolution of leaf area of plants under treatment effects.

3.7. Yield

The greatest yield was obtained in T3 treatment (6t / ha) while we obtained the lowest yield with treatment T1 (2t / ha) with respect to all other varieties (Figure 17). This is quite normal because the return is proportional to the leaf mass. We note that this yield is much lower than that obtained by Fotem [18] 7 t/ha when the crop is made between the 4th and 5th week after transplanting. This can be explained by the fact that *S. nigrum* grows crops, woods and clearings, rubble and other waste places, especially in shady, moist areas; meanwhile our experimental field was exposed to sunshine.

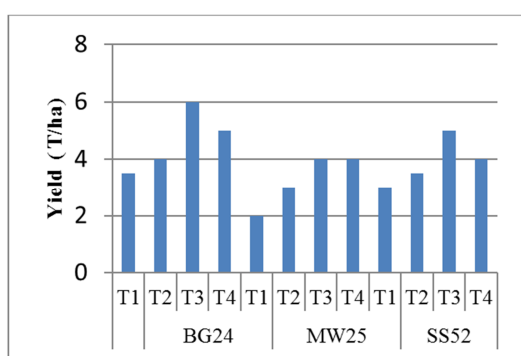


Figure 19. Evolution of the performance of black nightshade based on treatments and varieties N.B: Performance was evaluated in the first crop (4th week after transplant).

4. Conclusion

Determine all the pests that contribute to the infestation of *S. nigrum*, evaluate the effect of crop density on insect infestation, evaluate the effect of culture density on the

growth and yield are the three research areas which were the subject of our study.

The results obtained and presented here show that in general, *S. nigrum* is strongly attacked by three types of insects: aphids, black ants and whiteflies. The citation order marks the order of importance of the attack. Indeed, when the culture of its large densities (random or 20cmx20cm), *S. nigrum* is subject to many attacks. It notes from these experimental devices, a large number of infested plants and leaves. Culture density positively influences certain growth parameters (collar diameter, number of leaves, length of the rod and leaf area) and the yield of all varieties studied. Large densities (T1 and T2) and low density (T4) significantly drop leaf yield among all varieties considered. T3 treatment (30cm x 30cm) could be recommended to farmers to improve on farming techniques and significantly increase performance.

S. nigrum deserves to be highlighted because it is an excellent leafy vegetable and a major source of income for many vulnerable populations. After this study, we can say that much more is needed to find the biological characteristics of vector transmission of insects that infest the studied varieties and strategic fight against these pests.

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