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ACTION OF THE COMBINATION OF ALTERNARIA ALTERNATA AND NEOCHETINA EICHHORNIAE ON GROWTH PARAMETERS OF THE WATER HYACINTH IN A CONTROLLED ENVIRONMENT

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ABSTRACT

Alternaria alternata and *Neochetina eichhornia* are pathogens of water hyacinth which is a major invasive weed on our water ways in the south of Benin. We tested these two agents in a controlled environment on association water hyacinth. The device is made of four treatments with seven replicates .The association is made of two pairs of *Neochetina eichhornia* and *Alternaria alternate* with different sporulations 10^6 sp/ml, 10^7 sp/ml, 10^8 sp/ml, 10^9 sp/ml, 10^{10} sp/ml, 10^{11} sp/ml and 10^{12} sp/ml on water hyacinth for twelve weeks and a few days. The average values of the significant parameters of growth at the of the experiment as the weight with sporulation of 10^{12} sp/ml and of two pairs of *Neochetina eichhoenia* 18.80±0.35 g; those of leaves are 1.12±0.21 and those of buds are 0.80±0.13. Thus A. *alternata* is a potential as an agent of bio control of water hyacinth with $block_7$ T4 treatment.

KEYWORDS: *Alternaria alternata, Neochetina eichhorniae*, biocontrol, water hyacinth, Sporulation

INTRODUCTION

Alternaria alternata is a cosmopolitan fungus and was isolated from nearly all habitats (Ellis 1971; Domsch et *al* 1980;Farr et al. 1990, 2000; El-Morsy et *al*. 2000;Guoet et *al.* 2000). This optimal pathogen was also isolated from water hyacinth by several scholars in the world. The fungus causes diseases symptoms of (spots and lesions) mainly on the leaves and less severely on stolons, which eventually led to the death of the plant. Already the fungus has been described as a pathogen of the plant in Austrlia(Galbraith and Hayward, 1984) Egypt (Elwakil et *al.* 1989, Shabana et *al.* 1995; El-Morsy 2004), Bangladesh (Bardur-ud Din, 1978) and India (Aneja and Singh, 1989). The introduction in some tropical countries of water plants for ornamental purposes resulted to an uncontrolled swamping, followed by rapid suffocation of the lake and the destabilization of aquatic ecosystems (Gospel 1987, Labrada and Fornasari 2003). This has caused resulted in considerable socio-economic and environmental losses, especially in developing countries such as Benin, where precisely the water resources are often far from abundant (Tongo, 1996).Therefore, the elimination of this negative impact caused by water plants in the tropics, the fore of things, has become a topical issue. Over half of the 20th century, the massive application of spores of specific pathogens such as organic herbicides has attracted scholar's attention. Investigations have been conducted on many microorganisms and others are being studied (Charudattan, 2001; Auld et *al.* 2003). *Neochetina eichhorniae is* a very right and very resistant species originated from of the curculioidae family, of beetles whose body is mostly covered with grey spotted scales, brown grown antennas and segments legs are reddish-brown. The muscle is thick and slightly curved in males and especially thins quite strongly curved in females. The male can reach about 3.2mm in length (excluding head) and the female can reach approximately 3.7mm in length. The hypothesis that hyacinth infestation has spread like a flu by absence of natural enemies and in the conditions of pollution of water courses in infested areas, could be formulated. A number of micro-organisms become pathogenic on the plant. *Cercospora Rodmanii*, *A. alternata* and *A. eichhorniae* studies have shown the possibilities of effective control of *Eichhornia crassipes* (Charudatta 1996; Badu et *al.* 2003; Shabana 2005). Studies have been conducted in controlled and natural conditions on some pathogens isolated on the hyacinth. The death of the plant was obtained a few weeks after spraying with *Acromonium, Zonatum, Alternaria eichhorniae and Cercospora rodmanii* as well as *Alternaria alternata*. Several herbicides are effective against the hyacinth and are applied by air or land treatment. However, the ability of translocation of chemical molecules of stolons in the other parts of the plant is a limiting factor for herbicides. The old plants would be less sensitive than younger. During these last ten years, this plant has caused environmental and hydro-agricultural crises in Africa (Dangno et *al.* 2004).Although the origin of the infestation of water hyacinth in the world is known at the beginning of the 20th century that of its current expansion is poorly understood. Biological control could be an interesting alternative to chemical control. Biological control of the hyacinth is developed in 1960 by the importation of insects from the Amazon basin of the Brazil. (Deloach et *al.* 1989) *Bruchi* and *Neochetina eichhornia* were involved and have given good results on the water hyacinth (Ajuonu O. et *al.* 2003). This struggle is based on the use of natural enemies of the plant with the aim of creating a permanent pressure on it. That is why the objective of this work is to see the effect of association of the *Alternaria alternata* fungus and insect *Neochetina eichhorniae* on some growth parameters of water hyacinth controlled environment.

MATERIAL AND METHODS

Material

The biological material that has been the subject of experiments consists of *Alternaria alternate, Neochetina eichhorniae* and water hyacinth.

Methods

Culture of water hyacinth

Eichhornia crassipes was cultivated outdoor in containers far from insect reassign facilities to prevent accidental infestation on the site of plant physiology and Environmental stress Laboratory at the University of Abomey-Calavi. Plants are fed periodically with dropping from poultry every two weeks.

Photo 1: ***Eichhorniae Crassipes*** **Cultivation (Ajuonu 2014)**

Breeding of the Natural Enemies of the Water Hyacinth

The culture of these species has been initiated by the IITA-Benin since December 1991

Photo 2: Production of ***N. Eichhorniae*** **(Ajuonu 2014)**

Selection of Plants and ***N. Eichhorniae***

Eichhornia crassipes plants used in our research were selected culture of picture1 bins. After washing to remove waste organic as well as aphids which had taken refuge ten plants we arranged in each of the 28 basins in plastic 59cm in diameter and 30cm deep. It was previously planted to a depth of 20cm in the soil in order to refresh the water and keep the leaves in their state of turgor. (Ajuonu and *al* 2009) we used six liters of water added 3ml of chemical fertilizer in each bowl culture. *N. eichhorniae* adults used in our tests have been collected according to their sex and then kept in boxes of Petri dishes in glass and fed of young leaves of daily water hyacinth.

Multiplication of the Fungus ***Alternaria Alternata***

The multiplication of the inoculums has been carried out in Petri boxes each containing nutriment medium PDA(Potato Dextose Agar) to which are added 5ul of a spore suspension of *Alternaria alternata*. The boxes have been then incubated at 25°c oven for three weeks.

Photo 3: ***Alternaria Alternata*** **in Culture on PDA (FATON, 2015)**

Experimental Device

The experimental device used is a complete random block with 4 treatments and 7 technical runs with different concentrations of the fungus such as 10^6 sp/ml, 10^7sp/ml, 10^8sp/ml, 10^9sp/ml, 10^{10}sp/ml, 10^{11}sp/ml et 10^{12}sp/ml and two pairs of insects as shown in the table1 below.

Table 1: Experimental Device of the Different Treatments (FATON, 2015)

Blocks	Treatments	Elements of Each Treatments
1	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants of E. crassipes with 2 pairs (2males+2females) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes with *10^6sp/ml of Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^6 sp/ml Alternaria alternata* in the basin
2	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants of E. crassipes with 2 pairs (2males+2females) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes with *10^7sp/ml of Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^7 sp/ml Alternaria alternata* in the basin
3	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants of E. crassipes with 2 pairs (2males+2females) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes with *10^8sp/ml of Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^8 sp/ml Alternaria alternata* in the basin
4	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants d'E. crassipes avec 2 pairs (2mâles+2femelles) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes *avec 10^9 sp/ml Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^9 sp/ml Alternaria alternata* in the basin
5	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants of E. crassipes with 2 pairs (2males+2females) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes with *10^{10}sp/ml of Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^{10} sp/ml Alternaria alternata* in the basin
6	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants of E. crassipes with 2 pairs (2males+2females) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes with *10^{11}sp/ml of Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^{11} sp/ml Alternaria alternata* in the basin
7	**T1**	Evidence With 10 plants of E.crassipes without *N.eichhorniae* in the basin
	T2	10 plants of E. crassipes with 2 pairs (2males+2females) de *N. eichhorniae* in the basin
	T3	10 plants d'E. crassipes with *10^{12}sp/ml of Alternaria alternata* in the basin
	T4	10 plants of E. crassipes with 2 pairs (2males+2females) of *N. eichhorniae and 10^{12} sp/ml Alternaria alternata* in the basin

Data on different plant growth parameters have been taken at the beginning and the end of experiments on the weight, number of leaves, spots grazing, and flower buds. These data are recorded every two weeks.

Statistical Analysis of the Data

The excel table has been used to capture and process the data that have been noted in the form of average value standard error. This table is used to plot curves. The collected raw data have undergone a transformation by the function inverse sine of the root square prior to analysis. The other raw data have been transformed by the function log(x+1). A factorial analysis of variance (factorial ANOVA) has been used to examine the differences between treatments for each studied parameter. The method of comparison of variable used is the Student Newman Keuls(SKN) test. The analysis are performed using SAS analytical software (version9.2).

RESULTS

The results of these tests present data taken on the different growth parameters of *crassipes* subjected to different treatments T1, T2, T3 and T4 as shown below consist of four blocks operative. Figures 1,2,3,4, 5, 6 and 7 represent the evolution of fresh middleweight of the plants during our experiments for the four blocks. The T1 are the evidence of all treatments that have a considerable growth. Average values are placed at the beginning of the experience and no significant difference are found between the treatments (P= 0, 1541; 0, 1431; 0, 1232; 0, 0083; 0, 0526; 0, 0083; 0, 3331>0,005). Middleweight increased respectively 86.4g; 86.20g; 93.60 g; 90.50g; 86.5g; 88.4g and 88.8 g. For T2 treatment of each block, average weight at the end of the experience are respectively 68.5g; 63.30g; 72.42g; 66.10 g;67.00g; 68.10 and 66.10g. For T3 treatments of each blocks, the average weight obtained at the end of the experiment are respectively 66.8g; 63.3g; 59.30 g; 42.90g; 40.80g; 36.40 and 36.00g. For T4 treatment of each block, the average weights at the end of the experience are: 57.7g; 46.00g; 51.5g; 34.90g; 31.90g; 28.30g and 18.80g. At the end of the experience, treatments T2, T3 and T4 are very highly and significantly different from the evidence T1 to the 5% threshold (P<.0, 0001).

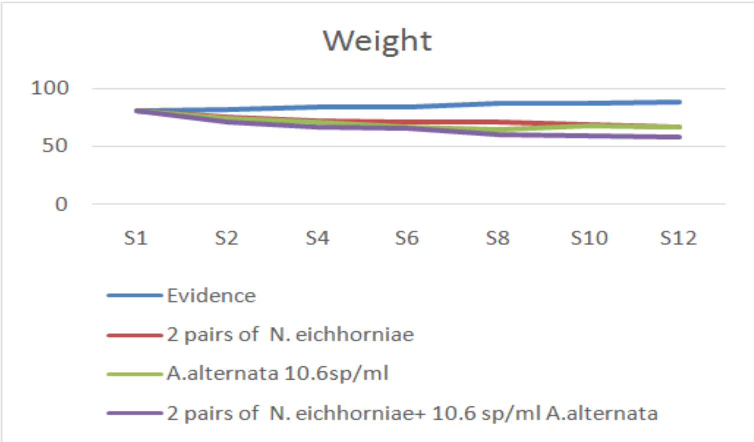

Figure 1: Average Evolution of the Water Hyacinth Plants

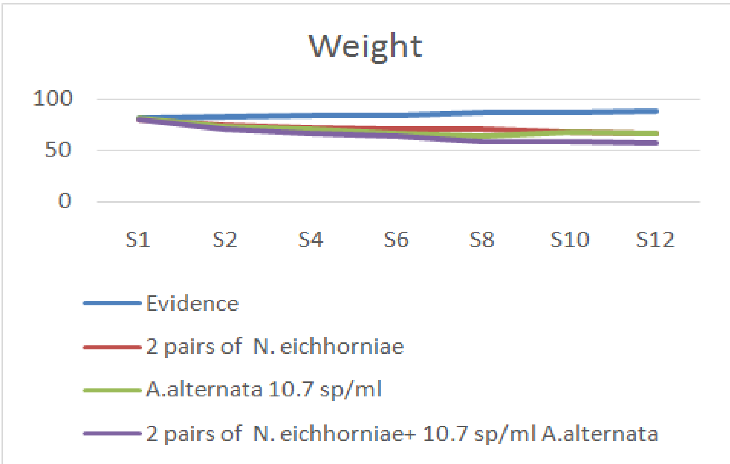

Figure 2: Average Evolution of the Water Hyacinth Plants

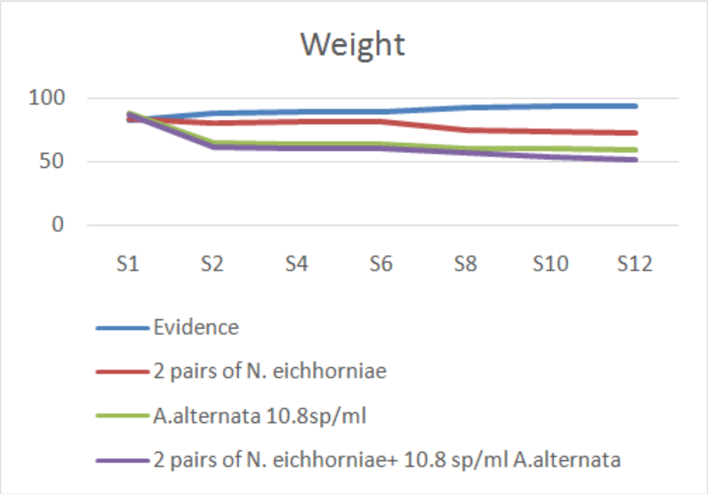

Figure 3: Average Evolution of the Water Hyacinth Plants

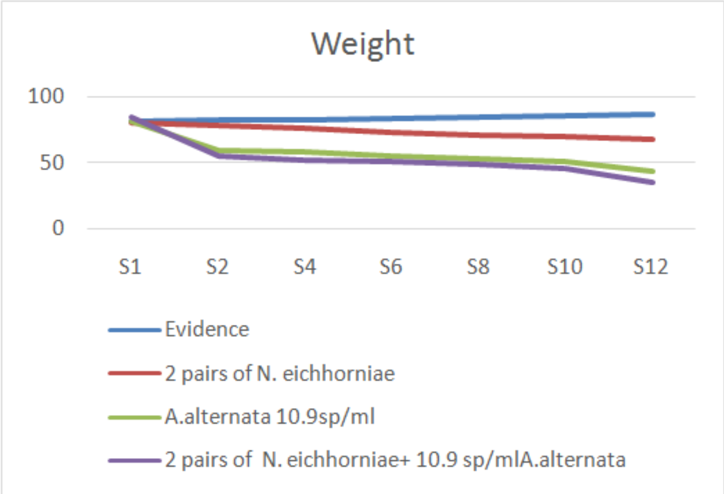

Figure 4: Average Evolution of the Water Hyacinth Plants

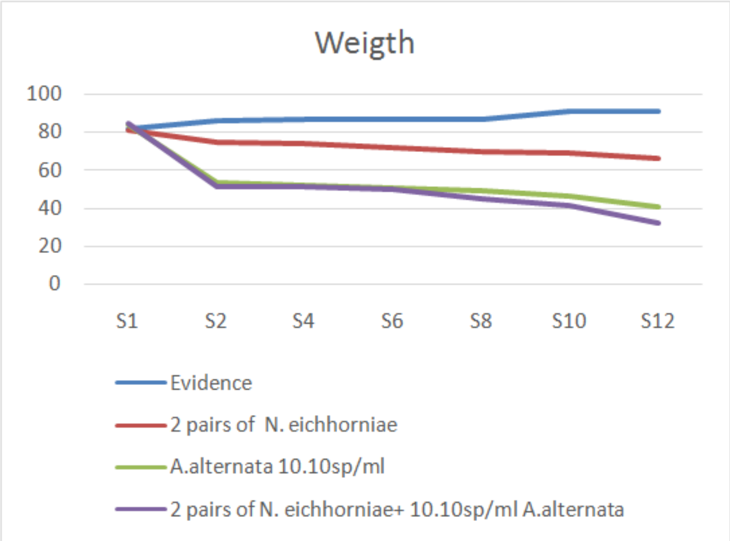

Figure 5: Average Evolution of the Water Hyacinth Plants

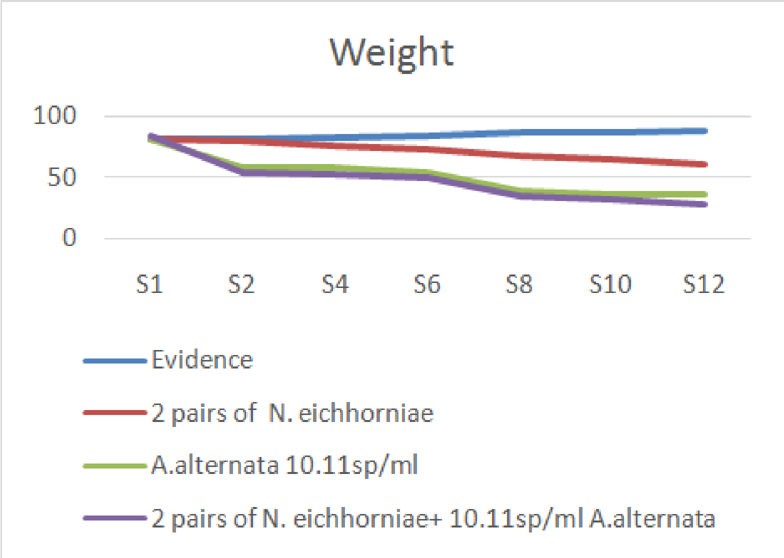

Figure 6: Average Evolution of the Water Hyacinth Plants

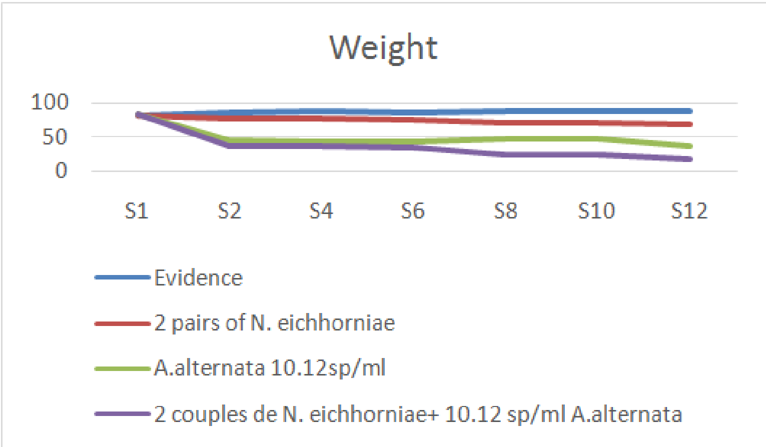

Figure 7: Average Evolution of the Water Hyacinth Plants

Figures 8, 9,10,12,13 and 14 represent the evolution of the number of leaves on the water hyacinth during our experiments for the seven blocks. T1 treatments represent the evidence. The number of leaves is counted on each plant at the beginning of the experiment, and no significant difference has been found between treatments. The number of leaves increased. Average values at the end of the experiment are respectively 9.4, 10.50, 9.30, 9.90, 9.30, 9.40 and 9.70. For treatment T2 of each block, the average values of number of leaves on plants are respectively 7.2, 6.3, 6.90, 7.00, 7.10, 7.20 and 6.40 at the end of the experiment. For the T3 treatment of each block, the average values of number of leaves on water hyacinth plants are respectively 5.30, 5.60, 6.00, 6.10, 7.1 and 6.5 at the end of the experiment. For T4 treatment of each block, the average values of number of leaves on water hyacinth plants are respectively 5.10, 5.00, 4.5, 4.00, 3.62, 1.3, and 1.1 at the end of the experience, the treatments T2, T3 and T4 of each block are highly and significantly different from the evidence T1 to the 5% threshold ($P<0.0001$).

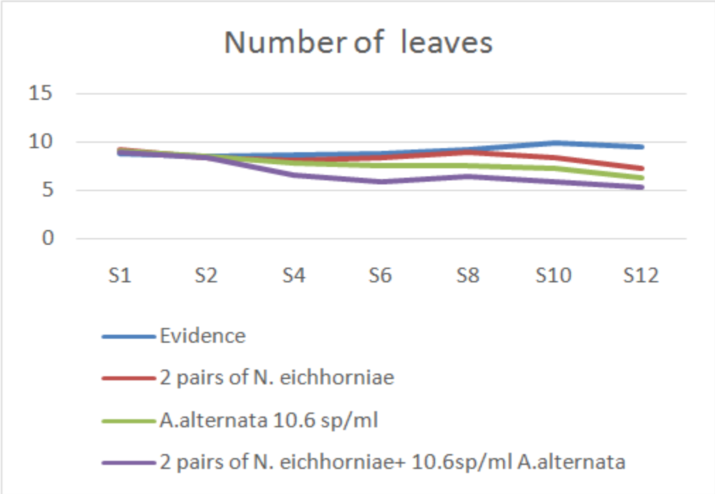

Figure 8: Average Grown of the Number of Leaves on the Water Hyacinth Plants

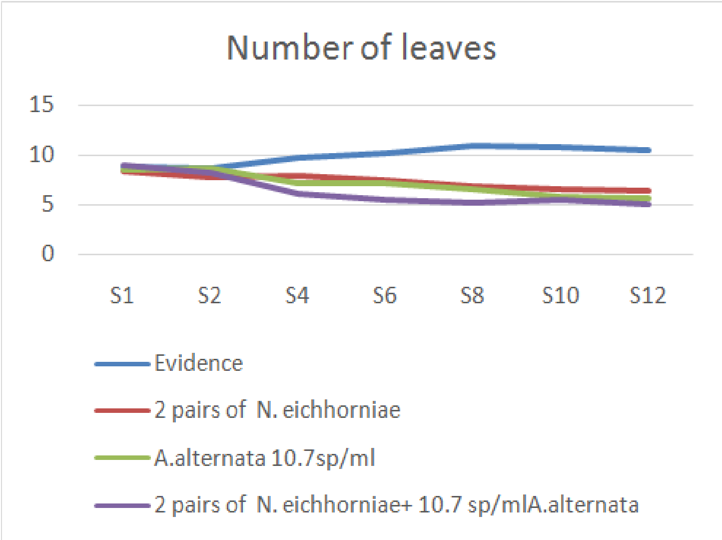

Figure 9: Average Grown of the Number of Leaves on the Water Hyacinth Plants

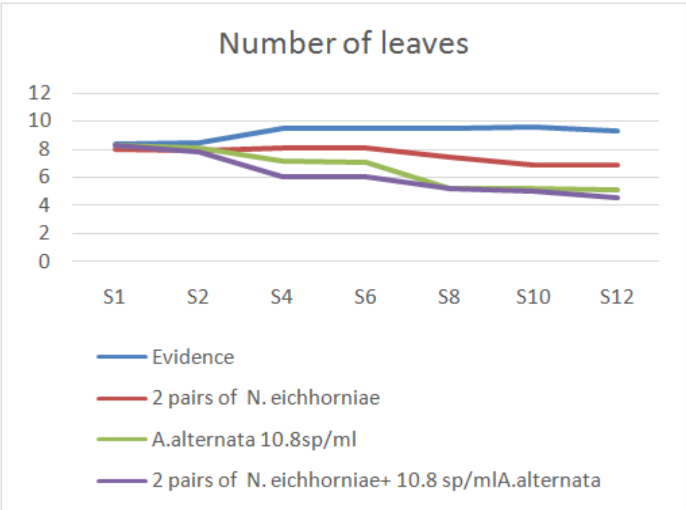

Figure 10: Average Grown of the Number of Leaves on the Water Hyacinth Plants

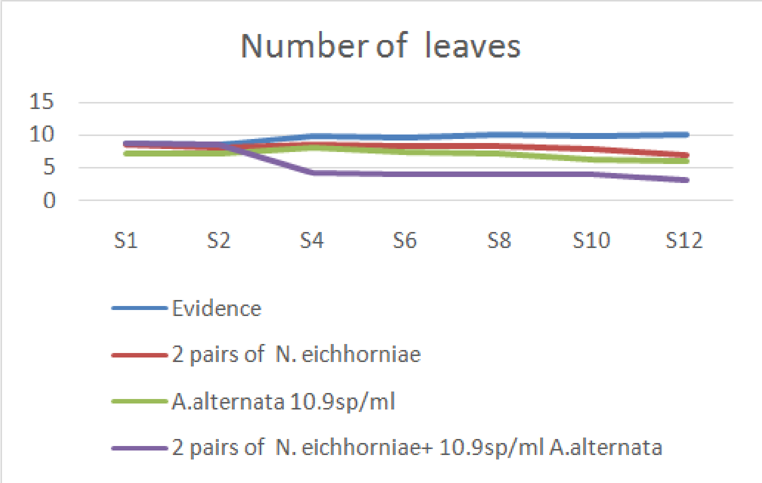

Figure 11: Average Grown of the Number of Leaves on the Water Hyacinth Plants

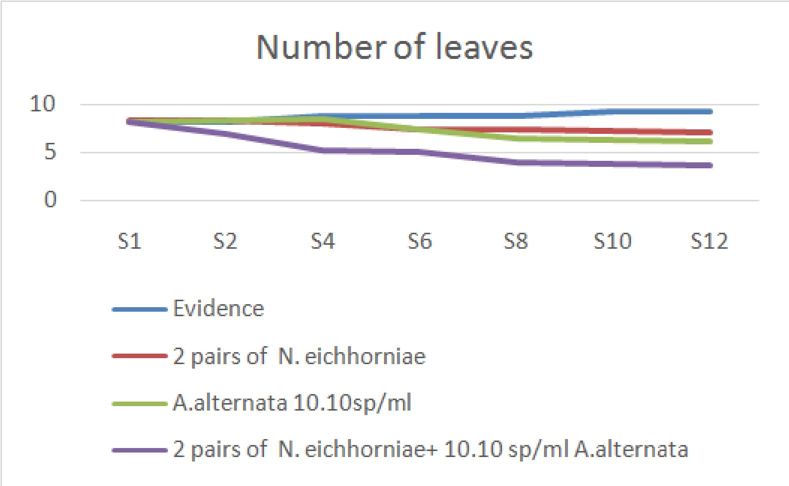

Figure 12: Average Grown of the Number of Leaves on the Water Hyacinth Plants

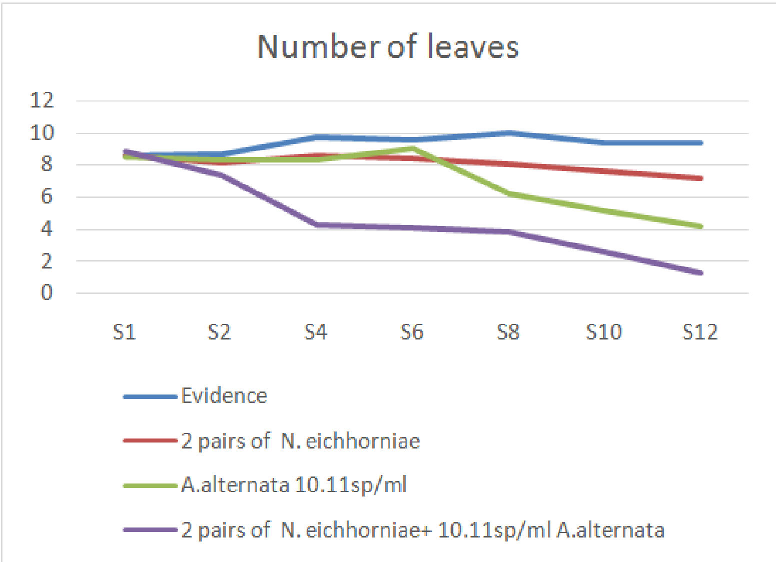

Figure 13: Average Grown of the Number of Leaves on the Water Hyacinth Plants

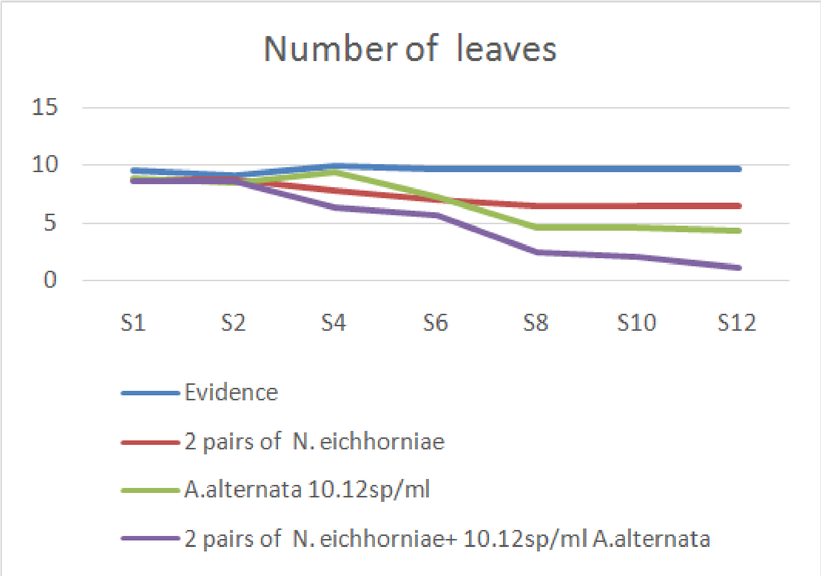

Figure 14: Average Grown of the Number of Leaves on the Water Hyacinth Plants

Figures 15, 16,17,18,19 and 20 represent the evolution of the number of dead leaves is counted on each plant at the beginning and at the end of the experiment. For treatment T1, we have not had a dead leaves on hyacinth plant during our experiences. For treatment T2 of each block, values of the number of dead leaves are respectively 2.80, 1.90, 1.90, 2.3, 1.82, 1.90 and 1.60 at the end of the experience. For treatment T3 of each block, the average values of the number of dead leaves on water hyacinth plants are respectively 2.2, 1.8, 2.4, 3.10, 5.1, 4.5 and 4.6 during testing or during the tests. For treatment T4 of each block, the average values of the experiment are respectively 2.00, 4.30, 4.6, 5.80, 6.70, 6.85 and 8.20 during our experiences. At the end of the experiences, the treatments T2, T3 and T4 of each block as highly and significantly different from the evidence T1 to the 5% threshold (P< 0.0001).

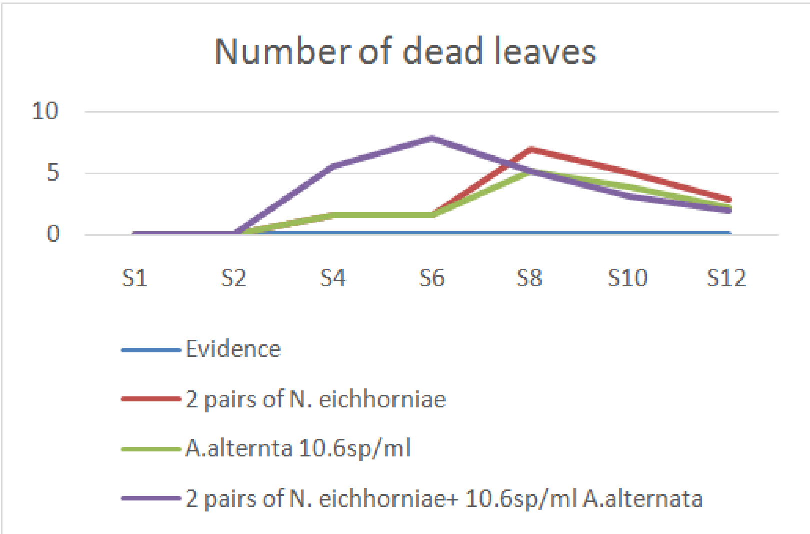

Figure 15: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

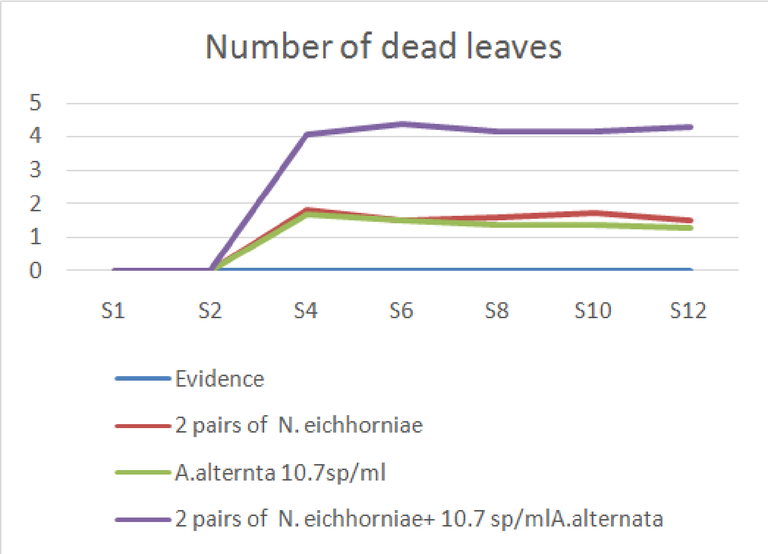

Figure 16: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

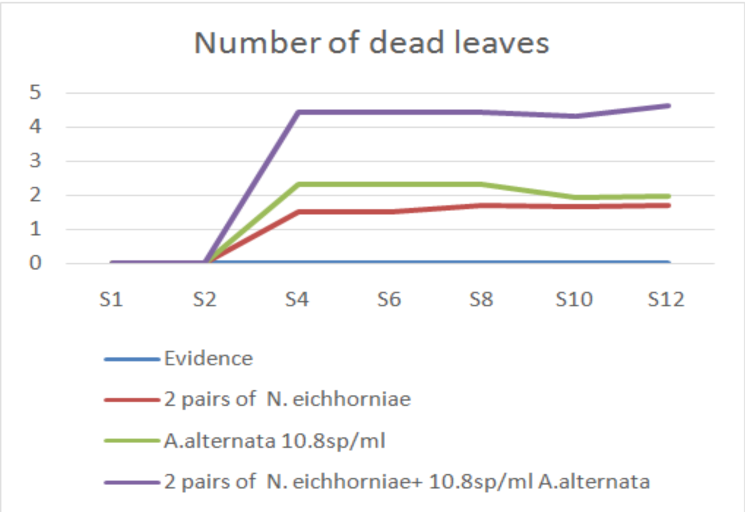

Figure 17: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

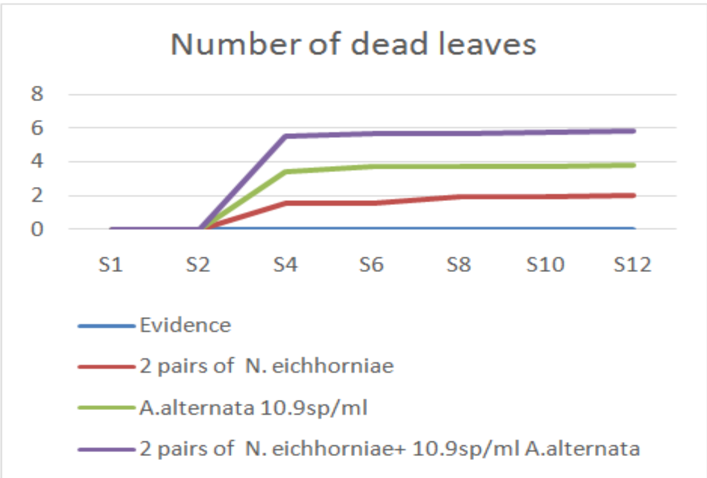

Figure 18: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

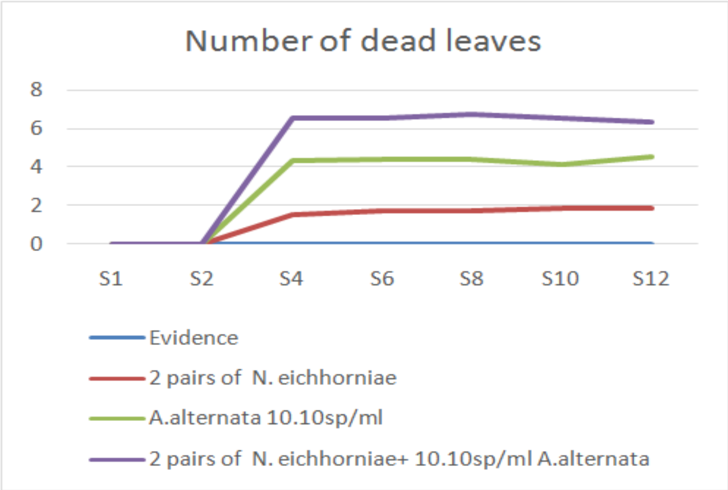

Figure 19: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

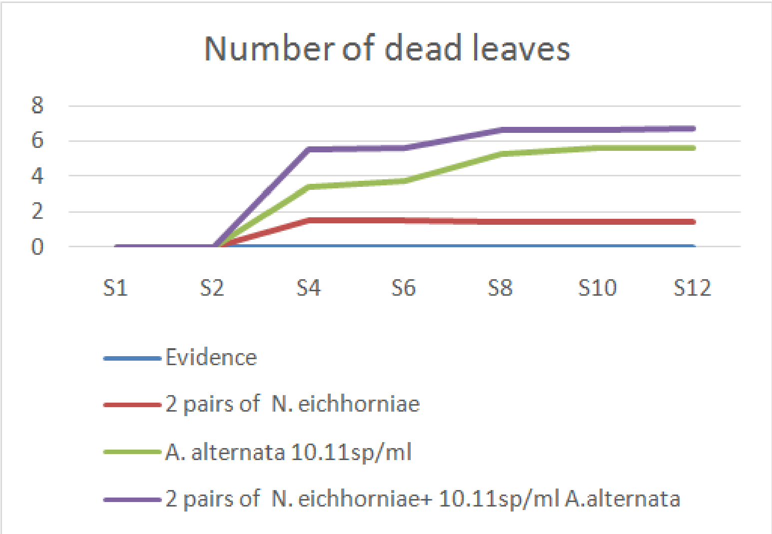

Figure 20: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

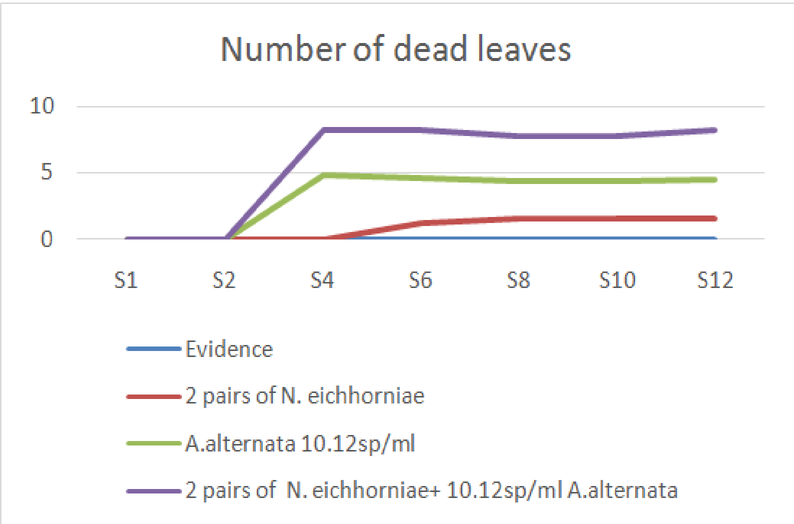

Figure 21: Average Grown of the Number of Dead Leaves on the Water Hyacinth Plants

Figures 22, 23 24 25 26 27 and 28 represent the evolution of the average number of leave scars the level of water hyacinth at during the trial period for the 4 blocks treatment T1 and T3 do not contain *N. eichlorniae* there foes no chatter at those levels. For T2 and T4 treatment, the average number of scars of chatter reached its peak in the second week from which the average number of scars declined until the eighth week. On the last day of our experiment, the average number of scars of chatter is very low for all of the two treatments in each block. The statistical analysis of these data reveals that the average of treatments T2 and T4 are highly and significantly different of 5% (P< 0.0001).

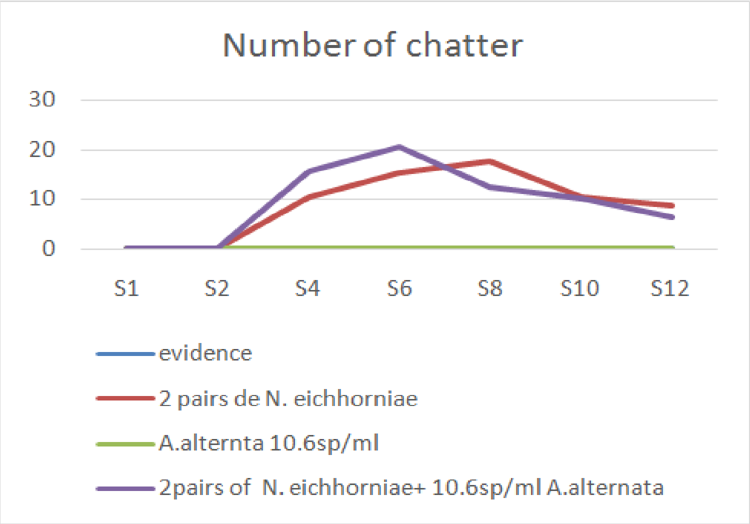

Figure 22: Average Change in the Number of Chatter on the Water Hyacinth Plants.

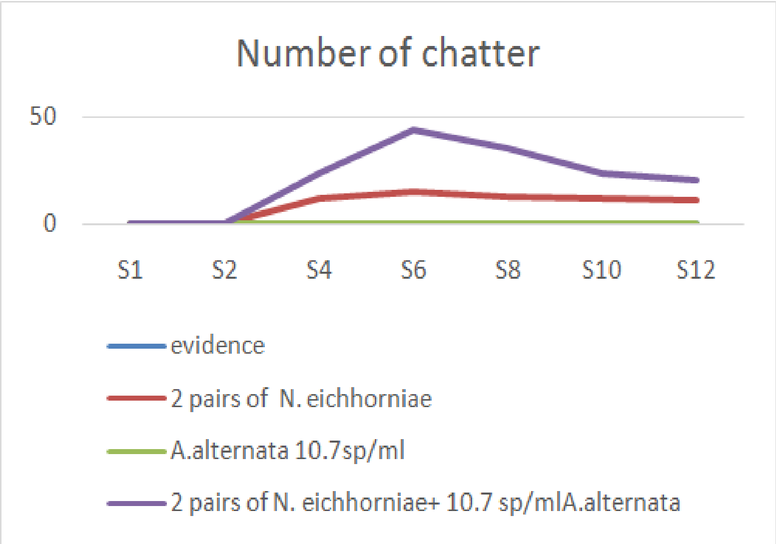

Figure 23: Average Change in the Number of Chatter on the Water Hyacinth Plants

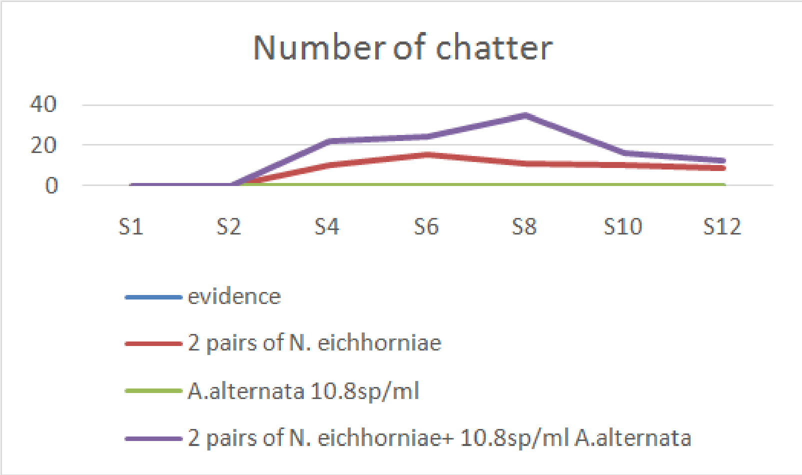

Figure 24: Average Change in the Number of Chatter on the Water Hyacinth Plants

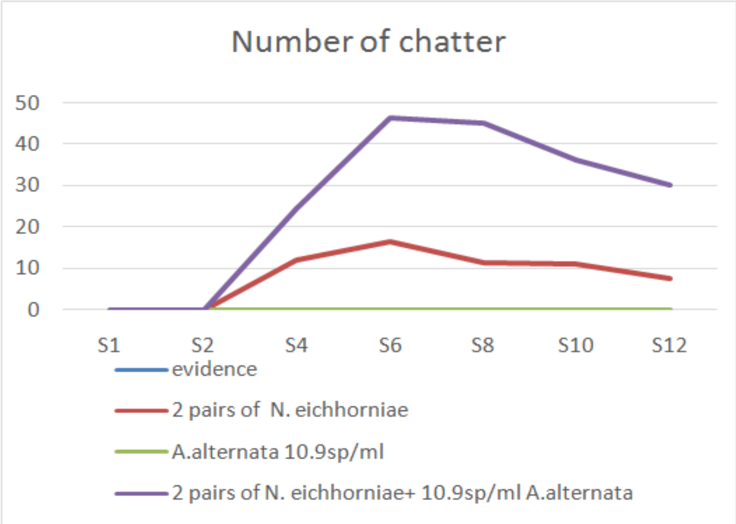

Figure 25: Average Change in the Number of Chatter on the Water Hyacinth Plants

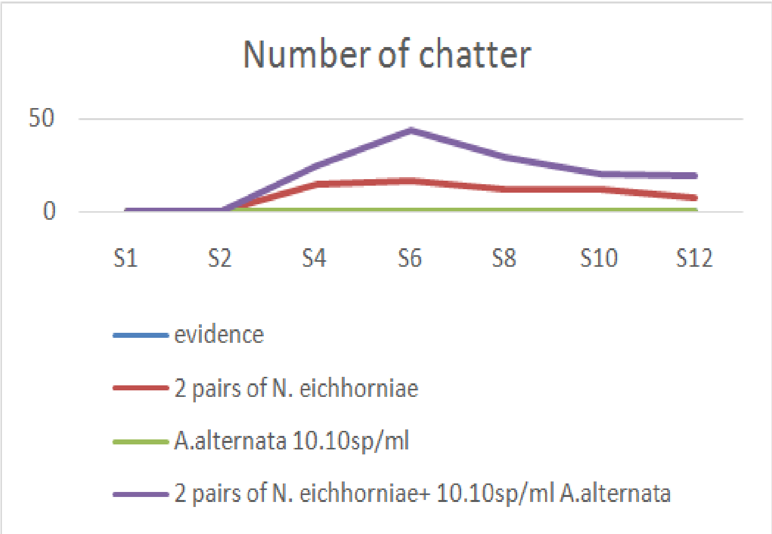

Figure 26: Average Change in the Number of Chatter on the Water Hyacinth Plant

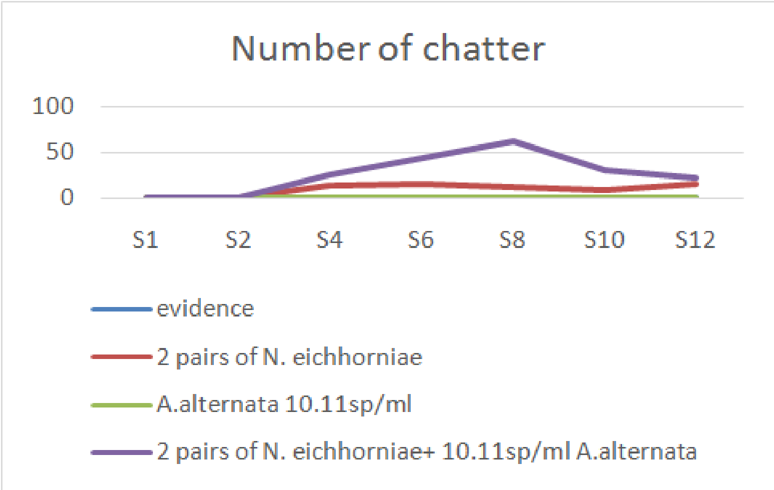

Figure 27: Average Change in the Number of Chatter on the Water Hyacinth Plants

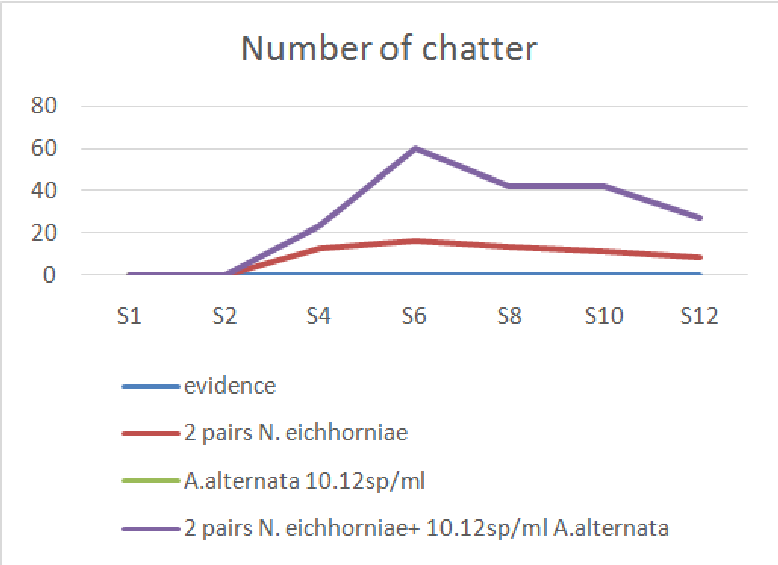

Figure 28: Average Change in the Number of Chatter on the Water Hyacinth Plants

Figures 29, 30, 31, 32, 33, 34 and 35 represent the averse evolution number of larval galleries on the water hyacinth during our experiments for the seven blocks Treatments T1 and T3 are without insects thus no larvae. For T2 and T4 Treatment, the average numbers of larval galleries was increased gradually during the experiment from as the second week and reach its peak at 6 weeks. From the seventh week, the average number of chatter has dropped gradually towards the end of the experiment. The statistical analysis of the data reveals a very significant difference between the treatment T2 and T4 averages and these of the treatment T1 and T3at the threshold of 5 %.

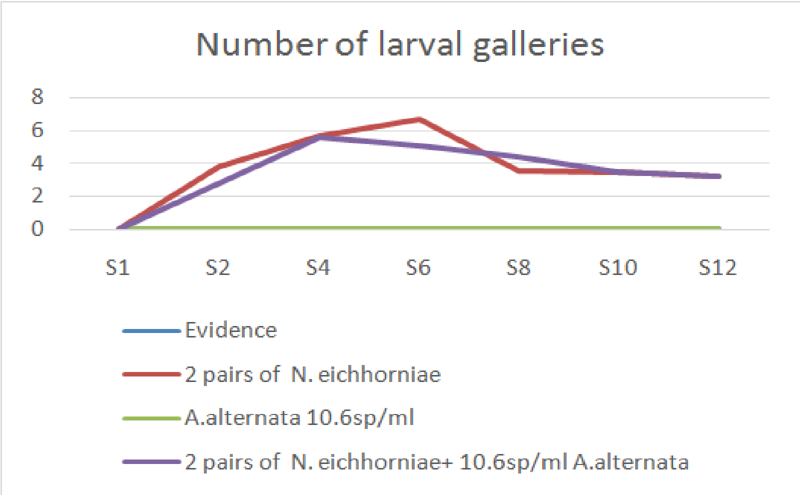

Figure 29: Average Change in Larval Galleries on the Water Hyacinth Plants

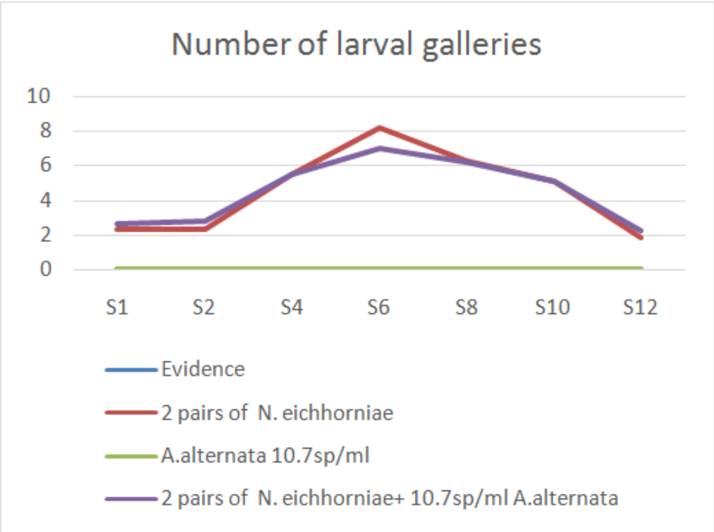

Figure 30: Average Change in Larval Galleries on the Water Hyacinth Plants

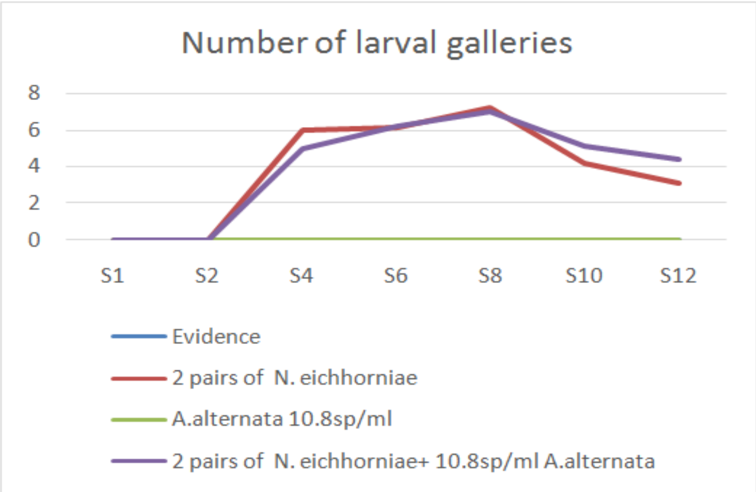

Figure 31: Average Change in Larval Galleries on the Water Hyacinth Plants

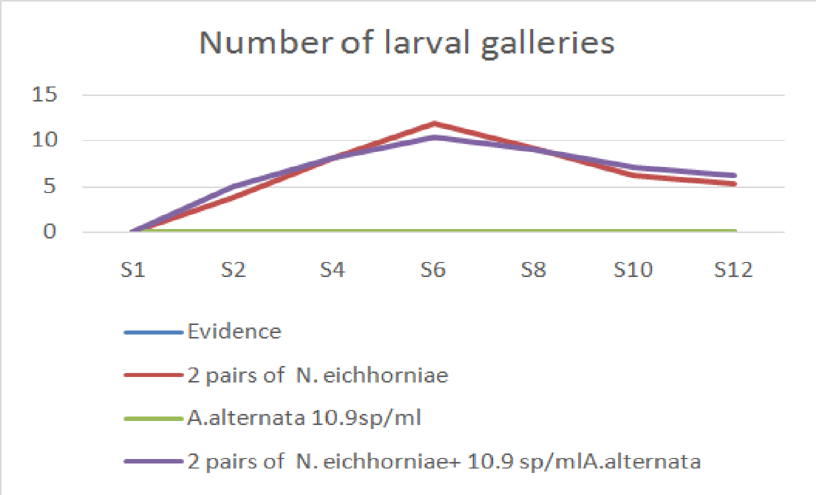

Figure 32: Average Change in Larval Galleries on the Water Hyacinth Plants

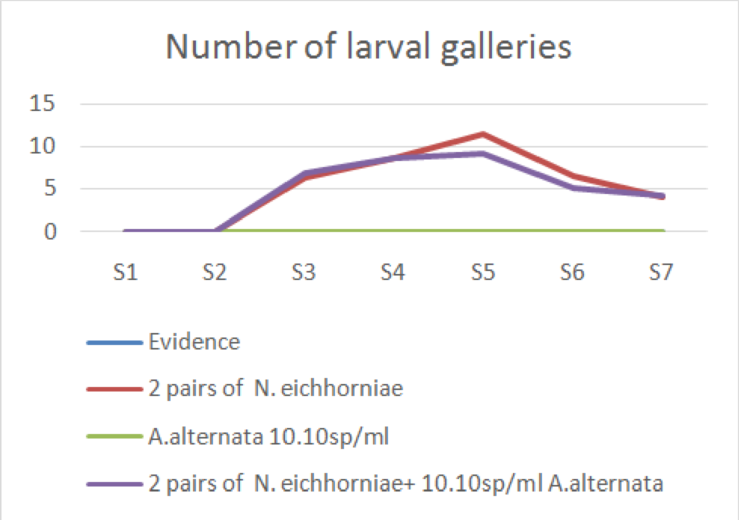

Figure 33: Average Change in Larval Galleries on the Water Hyacinth Plants

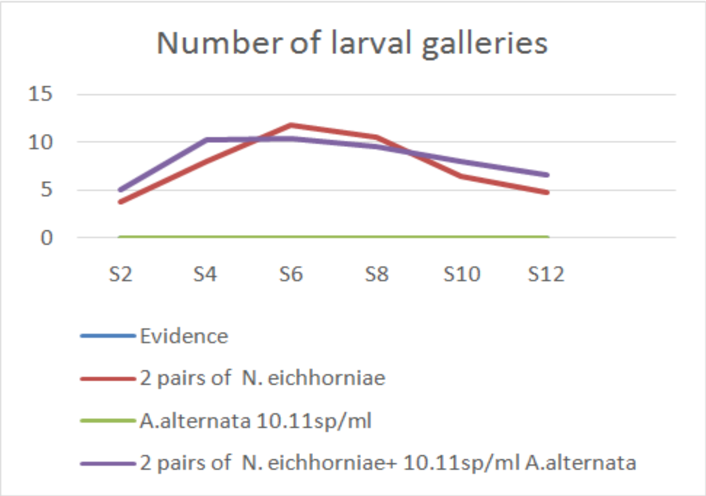

Figure 34: Average Change in Larval Galleries on the Water Hyacinth Plants

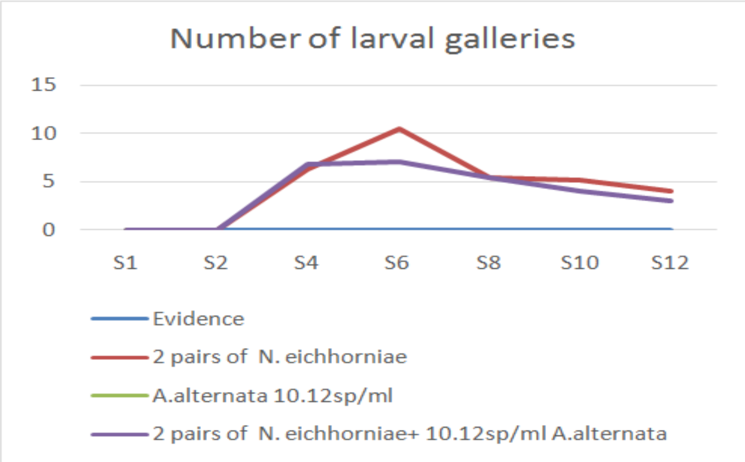

Figure 35: Average Change in Larval Galleries on the Water Hyacinth Plants

Average Number of Flower Buds on *Eichhorniae Crassipes* Plants

The tables 2, 3, 4,5,6,7 and 8 below present the average number of flowers on the water hyacinth plants during our experiment. The results show that a very small number of flower buds began to appear at the fourth week on plants of all seven blocks in general. At the end of the experiment, a very low number of plants are observed. For block 7, average values at the end of the experience are relatively low for T3 0.70±0.16 and T4 0.30± 0.15 treatments and an increase for T1 treatment 1.70±0.1.

Table 2: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of the Block 1

Traitements	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(Evidence)	0 ±0,00[a]	0 ±0,00[a]	1 ±0,00[a]	1,40±0,22[b]	2,30±0,39[a]	2,20±0,29[a]	2,40±0,33[a]
T2(*Neochetina eichhornia*)	0 ±0,00[a]	0 ±0,00[a]	1,40±0,20[a]	1,30±0,30[b]	1,0±0,33[a]	1,10±0,33[a]	1,0±0,33[b]
T3(*Alternaria alternata*10^{6}sp/ml)	0 ±0,00[a]	0 ±0,00[a]	1,20± 0,32[a]	1,10±0,33[a]	1,10±0,26[a]	1,00±0,21[a]	1,00±0,20[b]
T4(*Neochetina eichhornia +Alternaria alternata*10^{6}sp/ml)	0 ±0,00[a]	0 ±0,00[a]	0,75±0,20[a]	1,50±0,22[b]	1,30±0,21[a]	1,10±0,22[a]	1,0±0,24[b]
Probability	**0**	**0**	**<0,8250**	**<0,0914**	**<0,6247**	**<0,5160**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test

Table 3: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of Block 2

Treatments	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(Evidence)	0 ±0,00[a]	0 ±0,00[a]	1,60±0,16[a]	1,70±0,16[a]	1,80±0,16[a]	1,80±0,16[a]	1,90±0,16[a]
T2(Neochetina eichhornia)	0 ±0,00[a]	0 ±0,00[a]	1,80±0,26[a]	1,90±0,17[a]	1,20±0,13[a]	1,20±0,13[a]	1,30±0,13[a]
T3(*Alternaria alternata*10^{7}sp/ml)	0 ±0,00a	0 ±0,00a	1,60±0,20a	1,60±0,22a	1,60±0,16a	1,40±0,16a	1,30±0,16[a]
T4(*Neochetina eichhornia +Alternaria alternata*10^{7}sp/ml)	0 ±0,00a	0 ±0,00a	1,60±0,22a	1,60±0,22a	1,30±0,15a	1,20±0,15a	1,10±0,15[a]
Probability	**0**	**0**	**<0,9588**	**<0,4197**	**<0,7445**	**<0,8765**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test.

Table 4: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of Blocks 3

Treatments	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(Evidence)	0 ±0,00^a	0 ±0,00^a	1,70±0,16^a	1,70±0,16^a	1,60±0,16^a	2,30±0,16^a	2,30±0,16^a
T2(*Neochetina eichhornia*)	0 ±0,00^a	0 ±0,00^a	1,70±0,26^a	1,70±0,17^a	1,80±0,13^a	1,40±0,13^b	1,50±0,13^b
T3(*Alternaria alternata*10^{8}sp/ml)	0 ±0,00^a	0 ±0,00^a	1,70±0,20^a	1,70±0,22^a	1,30±0,16^a	1,20±0,16^b	1,20±0,16^b
T4(*Neochetina eichhornia +Alternaria alternata*10^{8}sp/ml)	0 ±0,00^a	0 ±0,00^a	1,60±0,22^a	1,60±0,22^a	1,30±0,15^a	1,60±0,15^b	1,10±0,15^b
Probability	**0**	**0**	**<0,0030**	**<0,0030**	**<0,2962**	**<0,0001**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test.

Table 5: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of Blocks 4

Treatments	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(Evidence)	0 ±0,00^a	0 ±0,00^a	1,60±0,16^a	1,60±0,16^a	1,70±0,16^a	1,70±0,16^a	1,70±0,16^a
T2(*Neochetina eichhornia)*	0 ±0,00^a	0 ±0,00^a	1,60±0,26^a	1,60±0,17^a	1,50±0,13^a	1,40±0,13^a	1,20±0,13^b
T3(*Alternaria alternata10^{9}sp*/ml)	0 ±0,00^a	0 ±0,00^a	1,50±0,20^a	1,40±0,22^a	1,40±0,16^a	1,30±0,16^a	1,20±0,16^b
T4(*Neochetina eichhornia +Alternaria alternata*10^{9}sp/ml)	0 ±0,00^a	0 ±0,00^a	1,00±0,22^a	1,10±0,22^a	1,10±0,15^a	1,30±0,15^a	1,00±0,15^b
Probability	**0**	**0**	**<0,5405**	**<0,3169**	**<0,0068**	**<0,3814**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test.

Table 6: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of Block 5

Treatments	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(Evidence)	0 ±0,00^a	0 ±0,00^a	1,70±0,16^a	1,60±0,16^a	1,40±0,16^a	1,60±0,16^a	1,90±0,1^a
T2(*Neochetina eichhornia*)	0 ±0,00^a	0 ±0,00^a	1,60±0,26^a	1,60±0,17^a	1,50±0,13^a	1,50±0,13^a	1,40±0,13^b
T3(*Alternaria alternata*10^{10}sp/ml)	0 ±0,00^a	0 ±0,00^a	1,50±0,20^a	1,40±0,22^a	1,30±0,16^a	1,30±0,16^a	1,10±0,16^b
T4(*Neochetina eichhornia +Alternaria alternata*10^{10}sp/ml)	0 ±0,00^a	0 ±0,00^a	1,20±0,15^a	1,30±0,12^a	1,10±0,15^a	1,00±0,15^a	1,0±0,15^b
Probability	**0**	**0**	**<0,1974**	**<0,8145**	**<0,9316**	**<0,7531**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test.

Table 7: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of Block 6

Treatments	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(TEvidence)	0 ±0,00[a]	0 ±0,00[a]	1,60±0,16[a]	1,60±0,16[a]	1,40±0,16[a]	1,60±0,16[a]	2,30±0,1[a]
T2(*Neochetina eichhornia*)	0 ±0,00[a]	0 ±0,00[a]	1,60±0,26[a]	1, 30±0,17[a]	1,20±0,13[a]	1,50±0,13[a]	1,20±0,13[b]
T3(*Alternaria alternata*10^11^sp/ml)	0 ±0,00[a]	0 ±0,00[a]	1,50±0,20[a]	1,40±0,22[a]	1,30±0,16[a]	1,20±0,16[a]	1,10±0,16[b]
T4(*Neochetina eichhornia* +*Alternaria alternata*10^11^sp/ml)	0 ±0,00[a]	0 ±0,00[a]	1,20±0,15[a]	1,30±0,12[b]	1,10±0,15[a]	1,00±0,15[a]	1,0±0,15[b]
Probability	**0**	**0**	**<0,5405**	**<0,3169**	**<0,9643**	**<0,5447**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test.

Table 8: Evolution of the Average Number of Flower Buds on Water Hyacinth Plants of Block 7

Treatments	Weeks						
	S1	S2	S4	S6	S8	S10	S12
T1(Evidence)	0 ±0,00[a]	0 ±0,00[a]	1,60±0,16[a]	1,60±0,16[a]	1,40±0,16[a]	1,60±0,16[a]	1,70±0,1[a]
T2(*Neochetina eichhornia*)	0 ±0,00[a]	0 ±0,00[a]	0,60±0,26[b]	0,80±0,17[b]	0,50±0,13[b]	0,50±0,13[b]	0,40±0,13[b]
T3(*Alternaria alternata*10^12^sp/ml)	0 ±0,00[a]	0 ±0,00[a]	0,50±0,20[b]	0,40±0,22[b]	0,30±0,16[b]	0,50±0,16[b]	0,50±0,16[b]
T4(*Neochetina eichhornia* +*Alternaria alternata*10^12^sp/ml)	0 ±0,00[a]	0 ±0,00[a]	0,20±0,15[b]	0,30±0,12[b]	0,10±0,15[b]	0,00±0,15[b]	0,30±0,15[b]
Probability	**0**	**0**	**<0,0045**	**<0,0021**	**<0,0012**	**<0,1110**	**<0,0001**

The averages on the same column affected by the same alphabetical letter are not statistically different at the threshold of 5% with ANOVA followed by student- Newman Keuls test.

DISCUSSIONS

In Africa and particularly in Benin, several individual of the weevil *N. eichhornia* who had been released in 1991 for the biological fight against water hyacinth had reduced the growth of the plant in some areas after eight years resulting in the reduction of the coverage of the surface of the take invaded by the weed from 5% to 78% (Ajvonu et *al* 2003) . A similar impact of Neochetina on the growth parameters of hyacinth was reported on Lake Victoria (Wilson et *al* 2005, 2007) and the United States (Center *al* 1989). *Alternaria alternata* is a pathogen of water hyacinth. An important result was observed on these growth parameters by El- Morsy and *al* 2004 where necroses were observed on leaves after a few weeks of treatment. Several growth parameters such as the weight and the leaves were weighed and counted at the beginning of the experiment on the one hand, the parameters as the number of dead leaves, the number of chatter, the number of larval galleries, were counted until the end of our experience. Physic-chemical parameters such as temperature, pH and dissolved oxygen are listed. In general the results of this study has been conducted under greenhouse with the loose of *Neochetina eichhorniae* and different sporulation *A.alternata*10^6sp/ml, 10^7sp/ml, 10^8sp/ml, 10^9sp/ml,10^{10}sp/ml, 10^{11}sp/ml and 10^{12}sp/ml on 10 plants of water hyacinth are consistent with the observations made by the authors cited above. The release of two pairs of *N. eichhorniae* crassipes significantly reduced after twelve week compared with the evidence on growth parameters. Inoculation of A. alternate on water hyacinth plants at these different sporulations, caused symptoms of the disease (spots and lesions) mainly on the leaves and less severely on stolons and finally gradually led to the plant death.

This is consistent with the result of Ajuonu et *al* 2003 in the field and Diop et *al* 2010 at the laboratory, who found that the weight of plants, one of the best of the impact of weevils on the biological control of aquatic plants in particular water hyacinth, had been significantly reduced in comparison to the weight of plants to the control treatment. For blocks 1, 2, 3, 4, 5,6 and 7, we got final weights, as average values respectively 86.40± 0.16g, 86.5 ±0.16g, 93.60± 0.16g, 86.5 ±0.20g, 90.50± 0.20g, 88.40± 0.16g and 88.21± 0.91g. It is the same for biomass of leaves, which has increased considerably at the end of the experience of which the averages values are respectively 9.40± 0.17, 10.50± 0.16, 9.30± 0.33, 9.90 ±0.17, 9.3±0.21, 9.4±0.016 and 9.97.021 for treatment T1. As regards the number of flower buds in bloom, from the fourth week avery small number appeared and increased towards the end of experience where we got a significant number respectively 2.40± 0.33, 1.8 ±0.20, 1.30 ±0.21, 1.70±0.16, 1.90±0.17, 2.3±0.13 and 2.30 ±0.21 for treatment T1. We have not counted of dead leaves at the end of the tests. These results show significant growth for the plant weight, leaves biomass and the number of flowers. For treatment T2, two pairs of *N. eichhorniae* are tested on ten plants of each treatment of the seven blocks. The average values of the weight obtained at the end of tests are respectively 68.50 ±0.16g, 63.30 ±0.70g, 72.40 ±0.16g, 67.0±0 0.73g, 66.10± 0.30g, 61.10± 0.40g and 60.10± 0.23g for the seven blocks. These results show a decrease in the weight of water hyacinth plants in comparison to the initial weight. These results are in conformity with that of (Center et *al.* 2005) who has obtained a reduction of five pairs of *N. eichhorniae* on water hyacinth plants. For the leaves, the average values obtained at the tests are respectively 7.20 ±1.15, 6.30 ±0.47, 6.90± 0.10, 7.00 ± 0.73, and 7.10± 0.34, 7.20± 0.26 and 6.50 ±0.22. These value obtained at the end show a decrease in the number of leaves on plants, so a reduction of surface coverage by these natural enemies of water hyacinth. *N. eichhornia*e. These same results are obtained by Ajuonu et *al.* 2003, on the reduction of the coverage of the lakes surface invaded by weeds from 5% to 100%. The average values of flowers number are respectively 2.30 ±0.33, 1.70± 0.21, 1.50 ±0.16, 1.50 ±0.16, 1.40± 0.16, 1.17 ±0.21 and 1.16± 0.16 for the seven blocks. These values show that there is a decrease in the level of plant growth because if there is not abortion, these flowers will become fruit. (Center et *al.* 2005) have observed the same phenomenon on the water hyacinth plants and reported that the impact of *N. eichhornia* reduces the ability of the plant to divert the resources required for the reproduction of flowers. Feeding scars on the leaves caused by grazing of *N. eichhornia* adults increased during the first two week and decreased as the number of adults decreases. This may be due to a lack of food and/or old age or death of the plant. Indeed, by feeding, adult laid eggs that hatches into larval 7-10 days later (Center, 1988) and it these larval which have caused damage observed on leaves from the second week and reached generally its peak in the sixth week for all blocks. These results are consistent to this of DeLoach and Cordo 1976 who studied the biology of *N. eichhorniae* and found damage on hyacinth are mainly due to the larval stage of *N. eichhorniae* leaving to mortality of the leaves. For treatment T3, only the fungus A. alternate is tested on water hyacinth plants at different sporulation. The average values obtained at the end of the experience are respectively 66.80± 0.13g, 54.50 ±1.13g, 59.30± 0.15g, 42.90± 0.56g, 40.80 ±0.13g, 36.40± 0.42g and 33.00± 0.29g.These results indicate that *A. alternata* has caused considerable damage on growth organs of the plant.What did decrease of the weight during the experiment. A better result is obtained for sporulation of 10^{12}sp/ml on the plants until the middle weight weighed initially passed from 82.90 ±0.96g to 36, 00±0, 29 at the end of the experiment. These results are confirming those of Mohan Babua et *al*. 2002, 2003a, b, c) that *A. alternata* reduces considerably the weight of water hyacinth at an important sporulation. Regards the leaves as early as the second round, sixth and fourth week respectively, a small number of necrotic spots and extensive damage began to appear on the leaves of all treatments, the peak is quickly obtained for sporulation of 10^{12}sp/ml similar results are obtained by El-sayed M El-Morsy and *al* 2004 who have observed the same leaf spots with brown center, lesions on the leaves and the death of the leaf after 15, 30 and 60 days. As for T4 treatment, a combination of *N. echhornia* and *A. alternata* to different sporulation are tested. The average values of the plants weight at the end of the tests are respectively 57.70 ±0.15g; 46.00± 0.68g; 51.50± 0.16g 34.90± 0.52g; 31.90 ±0.21g; 28.30± 0.33g and 18.80± 0.29g. These results indicate that weight was significantly decreased when comparing the four treatments. The association of the fungus and weevils acted seriously on the weight of the plants until it has passed with an average weight of plants 82.20 ±0.46g to 18.80± 0.29g with a sporulation of 10^{12}sp/ml. for the leaves, the same effects are observed where it rose by an average 8.60.±0.20 to 1.12± 0.21 after 12 weeks of treatment with sporulation of 10^{12}sp/ml. As the flower buds which are determinants of grown, they remained virtually absent during testing. The average values of these flown buds remained zero in the first two weeks for all treatments. It was until the fourth week that a small number of flower buds appear on water hyacinth plants. They are practically zero for treatment T4 *A. alternata* in association with *N. eichhorniae* at the end of experiment.

CONCLUSIONS

The aim of this work is to find more effective ways to improve the biological control of water hyacinth on our water ways. At the end of this work, the association of *N. eichhorniae* and *A. alternata* at different sporulation 10^6sp/ml, 10^7sp/ml, 10^8sp/ml, 10^9sp/ml, 10^{10}sp/ml, 10^{11}sp/ml and 10^{12}sp/ml on greenhouse tested water hyacinth, is effective in reducing the growth of *Eichhorniae crassipes* parameters. 10^{12}sp/ml of *A. alternata* and two pairs of N. *eichhorniae* have affected more significantly and more quickly growth parameters such as the weight of the plants, the leaf and flown buds.

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