



## Effects of biochar and mycorrhizae fungus on the larval developmental cycle of *Spodoptera exigua* H. (Noctuidea) and protection of *Allium cepa* L. Plants in farm

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### Abstract

The study was carried out in the Far-North Region of Cameroon, in order to measure the effects of biochar from neem leaves and mycorrhizal fungi on the developmental cycle of *Spodoptera exigua*, a foliar insect pest of *Allium cepa*. In breeding, the larvae of *Spodoptera exigua* were fed with onion leaves from seven treatments (T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>). Data analysis using Statgraphics software showed that onion leaves treated with biochar and mycorrhizae were less appetent (T<sub>1</sub> = 4.35%, T<sub>2</sub> = 4.33%) compared with leaves treated with mycorrhizae (T<sub>6</sub> = 7.58%) or treated with biochar (T<sub>3</sub> = 18.65%, T<sub>4</sub> = 18.81%). On the other hand, the leaves which had received neither biochar nor mycorrhizae were highly appreciated by the larvae (T<sub>0</sub> and T<sub>5</sub>) with a consumption > 34.76%. This feeding has some implications on the developmental cycle of *S. exigua* for the larval growth slowed down and the passage time from the larval stage to the emergence of the imago increased passing approximately over 12 days [T<sub>5</sub> = 12 ± 0.17 at T<sub>0</sub> = 12.10 ± 0.357] for leaves that received neither biochar nor mycorrhizae. This duration is about 16 days [T<sub>3</sub> = 16.50 ± 0.35; T<sub>4</sub> = 16.20 ± 0.283] in case biochar is used and 22 ± 0.436 days in case of the mycorrhizae (T<sub>6</sub>); and about 23 days when the leaves received both mycorrhizae and biochar [T<sub>1</sub> = 23 ± 0.46; T<sub>2</sub> = 23.50 ± 0.349]. The lethality due to these treatments is more marked in T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> treatments with a rate > 70%. This rate is 20% in biochar-based treatments (T<sub>3</sub> and T<sub>4</sub>) and < 10% in T<sub>0</sub> and T<sub>5</sub> treatments. In the field, the results showed that female *S. exigua* prefer untreated host plants (T<sub>0</sub> and T<sub>5</sub>) to lay their eggs. The highest infestation rate is observed in T<sub>0</sub> treatment (28.87%) and T<sub>5</sub> (27.82%). Biochar and mycorrhizae stimulate onion plants to synthesize secondary substances that are harmful to larvae and repellent to *S. exigua* adults.

**Keywords:** effect, biochar, mycorrhizal fungus, cycle, *Spodoptera exigua*, protection, *Allium cepa*.

## Introduction

The cultivation of onion is well adapted in the Soudano-Sahelianzone. The Far North Region of Cameroon is a large zone where onion is largely produced, and its economic importance is undeniable in this region. Onion is grown in dry season thanks to irrigation techniques. Yet, agricultural undertakers of this sector come across several problems: that of its hydric supply for the availability of water varies in this zone (Moustier and d'Arondel, 1994). Some authors pointed out some significant changes in the hydric equilibrium and the photosynthesis of onion crops, beans crops and cotton crops in salinity conditions (Gale et al., 1967) due to the misuse of chemical inputs and pesticides. They also encountered the problem insect pests for every year, insect pests use up (infest) averagely 10% of the vegetal production in natural systems and are responsible for 15% harvest reduction (or loss) in the world (Schultz, 2002). For their subsistence in this environment, plants have developed along with their evolution, different strategies to reduce or minimise damage caused to them by their natural enemies and especially phytophagans. *Spodoptera exigua* is a Lepidoptera foam of the *Noctuidea* family which uses *Allium cepa* plant as its host during the laying and the development of its larvas. The female of *Spodotera* chooses the best *Allium cepa* plant that can supply its offspring with qualitative food health assurance for their developmental cycle. *S. exigua* larvas are voracious phytophagans which, a few hours after hatching, pave their way and stay in the onion's leaves lumen in order to eat its parenchyma during the larval stage; then the pre-nymphal larva falls down for its nidation before the emergence of the imago. The damage made on the onion plants by these pre-nymphal larvas have some drawbacks on the production and consequently on the incomes of agricultural undertakers in the said-sector of activities. An infection rate (70%) is noted on the untreated farm. In order to reduce the use of pesticides, which are not harmful for the consumers' health, and in order promote a biological agriculture, we decided to carry out a study on the effect of biochar from dead leaves of neem (*Azadirachta intica*) and/or those of mycorrhizal fungus of the genus *Glomus* on the developmental cycle of *S. exigua* and on the protection of *Allium cepa* plants in farm.

This paper is especially out to measure the anti-appetence of onion leaves for larvas proportionally to the treatments applied; to measure effect of biochar and/or mycorrhizal fungus on the developmental cycle

of *S. exigua* (stating the transitional duration from one stage of the developmental to the other; stating the death rate during this period); and to measure the infection rate of plants by larvas in relation to the treatment applied.

## Materials and Methods

### Localisation of the zone of study

The study was realised in the Far North Region exactly in Diamare Division and precisely in Subdivision of Maroua 1<sup>er</sup>. The experimental device for the collection of the treated leaves had been realised in a site of IRAD in Meskine, a suburb of Maroua city (Figure 1). It is about 7 Km far from Maroua (longitude 14° 14' and 14° 15' East and latitude 10° 32' and 10° 33' North). Its relief is characterised by a plain with argillic and silty soil favourable for agricultural activities. Meskine is one of the main vegetable gardening basins of the Far North Region. The main sociologic components of Meskine consist in majority of Guiziga and Mofu peoples. There also live Tupuri, Massa, Fulbe, Moundang, etc. Because of this ethnic diversity, there is a mixture of religions such as animism, christianism, and islam. The socio-economic activities are essentially based on agriculture (vegetable gardening sector: vegetables, sweet potatoes, onions, carrots, cabbage, etc.), animal husbandry (small ruminants, pigs, bovines, fowl.) and small trades.

### Methods

In order to do a biological agriculture and to protect the nature altogether, we chose biochar made from dead leaves of neem (*Azadirachta indica*). This biochar is obtained by pyrolysis. We made this choice because biochar has an insect repellent capacity and its phosphoric content is a fundamental element for a good production of bulbs of onion. In addition to the biochar, mycorrhizal inoculum of the genus *Glomus* is used. The mycorrhizal inoculum fungus that develops symbiotic relationship with the host plant by providing it with phosphor from the soil; and in turn, it draws synthesised carbonate substances from the host plant.

Onion plants with *S. exigua* eggs were isolated in farm by means of thin-mesh sieve bags. Once the larvas hatched, the leaves containing them are collected back in jars covered by thin-mesh sieve and brought to the laboratory for breeding. The breeding was made in insectaria. Each insectarium is internally covered with foam; this foam is imbibed of water to maintain the

wetness of the place and to serve as nidation substrate during the nymphal stage of the developmental cycle of *S. exigua*. The harvested larvae were subdivided into populations of ten (10) in each insectarium stamped with types of leaf treatments disposed to feed them and cover them with thin-mesh sieve. In every interval of two days, 150g of leaves is put in every insectarium to feed the larvae up to the nymphal stage whereby they stop eating. The leftovers are weighed in order to determine the mass of consumed leaves. During this stage, we jotted down the transitional duration from the status of larva to nymph and from nymphal stage to imago on the one hand, and the death rate after the treatments on the other hand; this in order to account for or to state the behaviour of female adults in their selection of host plant on which the eggs will be laid. We randomly put an experimental device of seven blocks in farm. Each block is made up of seven compartments of 2x2m representing the seven treatments applied to the plants: T<sub>0</sub>= biochar (-) mycorrhizae (-) NPK(-); T<sub>1</sub>= biochar (+) mycorrhizae (+) NPK(+); T<sub>2</sub>= biochar (+) mycorrhizae (+) NPK(-); T<sub>3</sub>= biochar (+) mycorrhizae (-) NPK(+); T<sub>4</sub>= biochar (+) mycorrhizae (-) NPK(-); T<sub>5</sub>= biochar (-) mycorrhizae (-) NPK(+); T<sub>6</sub>= biochar (-) mycorrhizae (+) NPK(-). Each compartment contained 9x9 onion plants. We marked in a random way one compartment representing a specific treatment in each block. As such, we pick out the number of plants infected by the larvae in order to report infection rate of plants with respect to the treatments and to establish a correlation between the mass of consumed leaves in breeding and rate of infection of plants in farm.

Epidemiology is the study which deals with the development of a population of insect pests within a host population (grown plants on a parcel or an orchard). All the plants infected on the experimental

setting were counted in order to calculate the infection rate of the insect pest in the farm and by each treatment.

Finally, we took some leaves containing eggs of the insect pest that we brought to the laboratory for breeding; this with intention to identify this lepidopteran and make an account on the effect of the different treatments applied to plants on its developmental cycle.

### Statistical analysis

The data analysis had been done by means of the software STATGRAPHICS Centurion XVII.I., and based on basic statistical data analysis (average, variance, type gap) and ANOVA.

### Results

#### Effects of treatments on the consumption of leaves

Table 1 show the effect of treatments on *S. exigua* larvae' appetite. We realised that leaves of the plants which had undergone the treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> were less appetent with respectively only 4.35%, 4.33% and 7.58% of consumed leaves and that the leaves of plants which had undergone treatments T<sub>3</sub> and T<sub>4</sub> were consumed at about 18.65% and 18.81% respectively. Contrarily, leaves from treatments T<sub>0</sub> and T<sub>5</sub> were most appreciated and consumed by *S. exigua* larvae at 34.76%. This differential preference of leaves of plants treated with biochar and/or not with mycorrhizae fungi would be due to the capacity to stimulate the production secondary substances which irritate and turn the leaves less appetent that these inoculums give the plants.

**Table 1: Mass of leaves eaten by *S. exigua* larvae in breeding**

Treatment	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
Total mass (g)	1200	2550	2550	1650	1650	1200	2400
Consumed mass (g)	418.47	110.94	110.46	307.52	310.39	417.11	181.85
Consumption%	34.87	4.35	4.33	18.64	18.81	34.76	7.58

However, the feeding quality and their availability have significant consequences on the duration of the developmental cycle of *S. exigua*. Table 2 presents the effects of treatments on the transitional duration from the larval stage to the nymphal one (larval duration) on

the one hand and from nymphal stage to the imago one (nymphal duration) on the other hand. There is significant difference for any treatment at a threshold of 5%.

**Table 2: Effects of treatments on the duration of the developmental cycle of *S. Exigua***

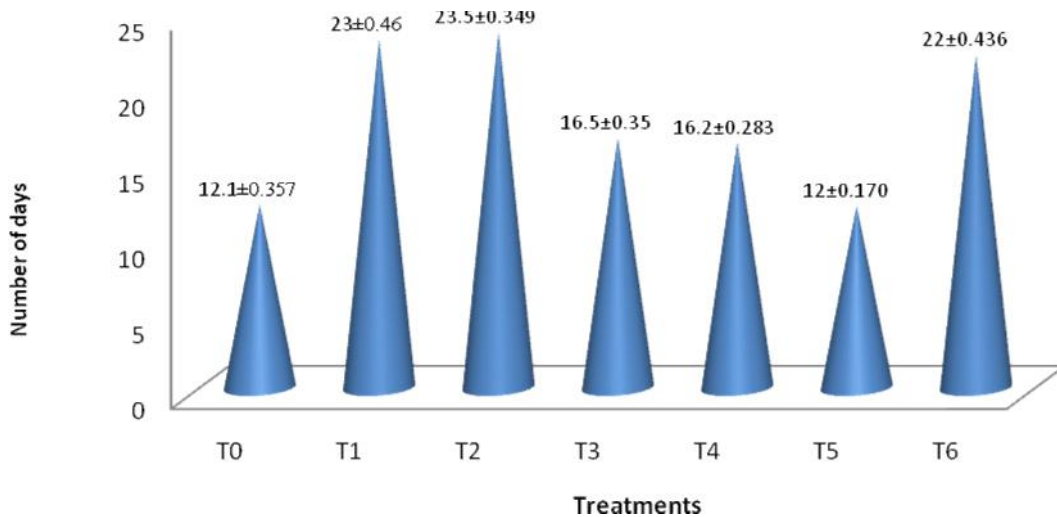
Treatments	Larval duration (D)	Nymphal duration (D)	Average totals
T <sub>0</sub>	7.40±0.418c	4.70±0.250b	12.10±0.357c
T <sub>1</sub>	16.50±0.412a	6.50±0.412a	23.00±0.460a
T <sub>2</sub>	16.70±0.274a	6.80±0.350a	23.50±0.349a
T <sub>3</sub>	11.40±0.322b	5.10±0.180ab	16.50±0.350b
T <sub>4</sub>	11.60±0.218b	4.60±0.218b	16.20±0.283b
T <sub>5</sub>	7.20±0.392c	4.80±0.326b	12.00±0.170c
T <sub>6</sub>	15.70±0.405a	6.30±0.391a	22.00±0.436a

Values of the same column followed the same letter are not significantly different (p< 0.05)

The period of transition from the larval stage to nymphal stage is higher for the larvae fed with leaves from treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub>. This time is set between 15.70±0.405 and 16.70±0.274 days. This period is very low in treatments T<sub>0</sub> and T<sub>5</sub> with respectively 7.40±0.418 and 7.20±0.392 days. As for treatments T<sub>3</sub> and T<sub>4</sub>, it extends respectively on 11.40±0.32 and 11.60±0.218 days. Taking into consideration the transitional period from the nymphal stage to the emergence of the imago, we found a duration of more than six (06) days in treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub>. Whereas in treatments T<sub>0</sub>, T<sub>4</sub> and T<sub>5</sub> the

duration is less than five (05) days. For the treatment T<sub>3</sub>, duration is 5.10±0.180 days.

Generally, the overall time of transition from the larval stage (hatching) to the emergence of the imago is about 12 days for *S. exigua*. Figure 1 displays similar results in treatments T<sub>0</sub> and T<sub>5</sub>. Meanwhile, there is about 4 days in treatments T<sub>3</sub> and T<sub>4</sub> with respective duration of 16.50±0.35 and 16.20±0.283 days. In treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub>, this increase is almost equal to the double of the normal duration (about 12 days).



**Figure 1: Period of transition from the larval stage to the emergence of the imago of *S. exigua***

**Effects of treatments on the life of larvae and nymphs**

It was equally observed that the feeding quality does not impact only on the duration of the developmental cycle of *S. exigua* but also on the life of the species. Table 3 accounts for the effects treatments on the survival of larvae and nymphs.

A high death rate was observed on larvae which had received treatments T<sub>1</sub> and T<sub>2</sub> with respectively 50% and 60%. This rate is inferior to 40% in treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>6</sub>; and 0% in treatments T<sub>0</sub> and T<sub>5</sub>.

As for nymphs, a high death rate was observed in treatments T<sub>1</sub> and T<sub>2</sub> respectively 80.50% and 75.14%. No death was observed in treatments T<sub>0</sub>, T<sub>3</sub> and T<sub>4</sub>; meanwhile there is 10% of death in treatment T<sub>5</sub>.

**Table 3: Effect of treatments on the survival of larvae and nymphs of *S. exigua***

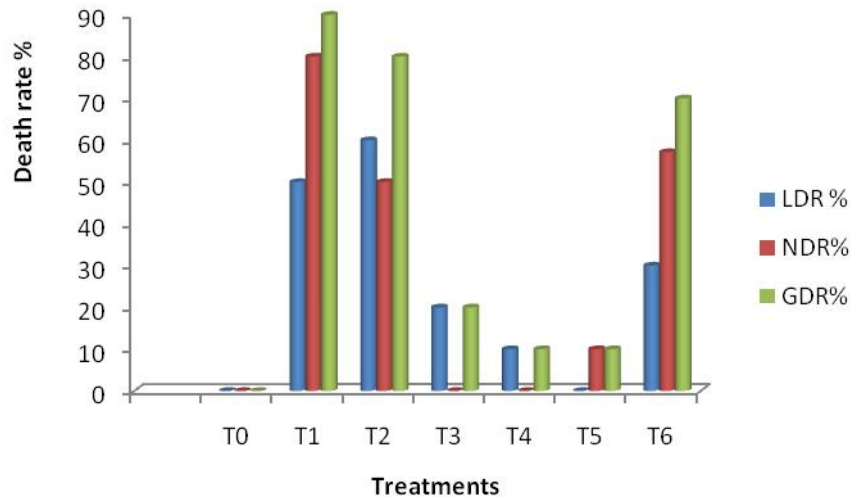
Treatment		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
Larvas	Alive	10	10	10	10	10	10	10
	Dead	0	5	6	2	1	0	3
LDR %		<b>0</b>	<b>50</b>	<b>60</b>	<b>20</b>	<b>10</b>	<b>0</b>	<b>30</b>
Nymphs	Alive	10	5	4	8	9	10	7
	Dead	0	4	2	0	0	1	4
NDR %		<b>0</b>	<b>80</b>	<b>50</b>	<b>0</b>	<b>0</b>	<b>10</b>	<b>57.14</b>
Imago %		<b>100</b>	<b>10</b>	<b>20</b>	<b>80</b>	<b>90</b>	<b>90</b>	<b>30</b>
GDR %		<b>0</b>	<b>90</b>	<b>80</b>	<b>20</b>	<b>10</b>	<b>10</b>	<b>70</b>

LMR= Larval death rate; NDR= Nymphal death rate; GDR= General death rate

**Effects of treatments on the larval development of *S. exigua***

Figure 2 explains that treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>6</sub> have lethal effects on the larval development of *S. exigua*

for it comes from this analysis that 70% of larvae fed with treated leaves have not reached the imago stage. In treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>, this death rate is inferior to 20% and nil in treatments T<sub>0</sub>.



**Figure 2: Death rate of larvae and nymphs of *S. exigua***

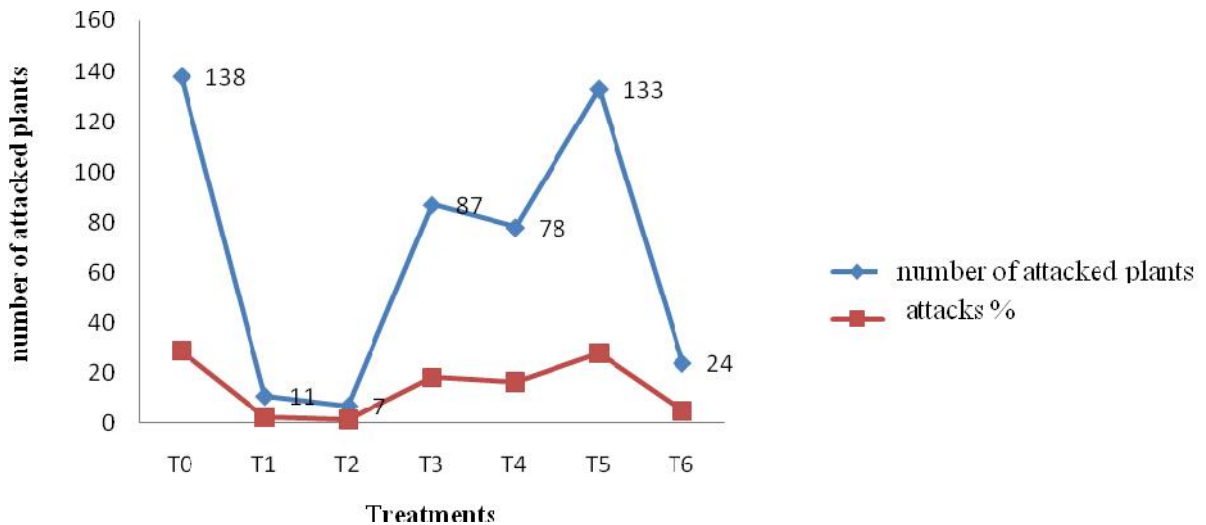
**Onion foliar attacks by *S. exigua* larvae' in farm**

Data analyses on the foliar attacks by *S. exigua* larvae in farm were jotted in table 4. In fact, it is all about plants which had been selected by female of *S. exigua* at laying moment. Female of *S. exigua* preferably chose plants which had undergone treatments T<sub>0</sub> and T<sub>5</sub> with respectively 138 and 133 infested plants, that

is more than 56% compared to plants that had undergone treatments T<sub>3</sub> and T<sub>4</sub> with about 34.52%. Less infested plants as they were not selected by the female *S. exigua* are only those which had received treatments T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> with respective infestation rate of 2.30%; 1.47% and 5.02% (Figure 3). There no significant difference among these three treatments.

**Table 4: Infestation rate of plants by *S. exigua* larvae**

Treatments	Number of attacked plants	Attacks %
T <sub>0</sub>	138	28.87
T <sub>1</sub>	11	2.3
T <sub>2</sub>	7	1.47
T <sub>3</sub>	87	18.2
T <sub>4</sub>	78	16.32
T <sub>5</sub>	133	27.82
T <sub>6</sub>	24	5.02
Totals	<b>478</b>	<b>100</b>



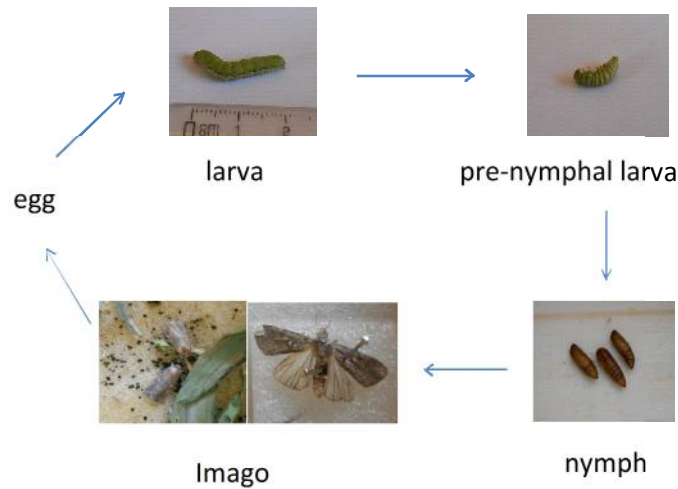
**Figure 3: Curve of plants infection by *S. exigua* larvae**

**Development of *S. exigua* larval cycle in laboratory**

*S. exigua* is a *Noctuidea* whose larval developmental cycle has duration of about 12 days: from hatching to the pre-nymphal stage, it takes 4 to 5 days (Figure 4). This noctuidea's period of the larval developmental

cycle can experience modifications due to ambient temperature and the type of food brought to the larvae as well as other physical factors. Dahms (1972 b) used the term antibiosis to refer to plants that had developed a resistance resulting in unfavourable effects on bugs biology (development and reproduction).





**Figure 4: Developmental cycle of the *Sporodoptera exigua* (Noctuidea)**

## Discussion

The cycle of larval development can undergo modifications in time and in space proportionally to the quality of food supplied to larvae, to ambient temperature and to physical factors. For the fact that leaves having undergone some treatments ( $T_1$ ,  $T_2$  and  $T_6$ ) were less appetent than others could be related the capacity of these treatments to help onion plants in realising the synthetisation of secondary substances which could have harmful effects on this insect pest. Breynays and Chapman (1994) stated that most of these products have an impact on the behaviour of a significant number of phytophagous insects through repulsion process or anti-appetency. Azadirachtin, one of the active substances of the *Azadirachta indica* (neem) has been proved as a powerful anti-appetent for *Schistocerca gregarcia* as well as a systemic deregulator of growth; generally non-toxic for vertebrates (Meinwald et al., 1978).

Dahms (1972 b) used the term antibiosis to refer to plants which had developed a resistance that brought unfavourable effects the development and reproduction of insects. Meinwald et al. (1978) reported that extracts of *Azadirachta indica* or lilas of Perse (neem)'s leaves of seeds, applied in foliar treatment or by incorporation in food, could have negative effects on the development of a certain number of insects. These observations corroborate the modifications viewed in the duration of the developmental cycle of *S. exigua* when larvae are fed with leaves from treatments  $T_1$ ,  $T_2$  and  $T_6$ . The same observations were made by Isman (1997) who, working on the aspect of insecticides of the neem, had put in isolation a substance called Azadirachtin which

has an inhibitive action on the growth and moult of insects and therefore their reproductive cycle.

Out of the observed cases of larval and nymphal death, no phenotypical anomaly has been observed on the adults. Dahms (1972 b) had frequently cited as evidence of antibiosis, instances of premature death at larval stage. Saxena et al. (1981 b) said that larvae' hatching at fifth (5<sup>th</sup>) moult of the striped pyrale of the rice, on plants treated with minimum of 12% of *Azadirachta indica* oil, led to the development of monstrosities at stages of larva/chrysalides. However, Van Dam et al. (2000) stated that young caterpillars of *Manduca sexta*, constrained to grow on tobacco plants the systems of which were previously induced, displayed a high death rate and the survivors experienced very slow growth.

The observed differences of foliar attacks rate in farm by the larvae (in relation with the applied treatments) could be due to the capacity of onion plants to synthesise substances of harmful effects on the survival, the development and the reproduction of *S. exigua*; this in presence biochar and/or the mycorrhizae inoculum. These effects are more noticeable when biochar and the mycorrhizas are applied simultaneously. These characteristics of plants which affect as such – particularly – the behaviour of the female adult insect when it tries to pave its way in a search for food, shelter or accommodation site to lay its eggs. These so produced substances by plants turn them less attractive or rather inaccessible to insect pests for they become less appetent, hindering as such the phenomena of colonisation of these plants by

insects (Bernays and Chapman, 1994). Pathak and Saxena (1979) indicated that, in replanted cultures of open farm, “non-preferred” varieties of crops are most of the times free of infestations and that, even when the insects are caged on on-preferred hosts, they tend to lay less eggs and to produce more reduced population compare to the situation when they are installed on sensitive hosts. These authors further underline that the presence of either an anti-appetent or an inhibitor of larval growth or that of an association of these two elements can provide the plants with resistance ability to insect pests.

In farm, we observed a low rate of foliar attacks by *S. exigua* in parcels treated with biochar and/or mycorrhizae compared to show parcels. It results from this observation that these substances impact on the behaviour of female *S. exigua* which chooses plants able to feed it with qualitative food for an optimal development of its offspring and its survival. Johnson *et al.* (1989); Rosenthal and Berenbaum (1991), Duffey and Stout (1996) and Royo *et al.* (1999) pointed out that most of secondary substances impacts negatively on the insect pests that directly affect parameters related to the fitness of phytophagans such as growth, development, reproduction and/or life expectancy.

The contribution of biochar/mycorrhizae in the synthesis of protective substances by plants themselves helped in the modification of the biological balance of *Spodoptera exigua* whose larva eats onion leaves. It is worth noting from our observations that these substances have effects on the biology of this insect pest. For our investigations helped us to point out on the one hand cases of mortality among the populations of larvas fed with treated leaves and on the other hand a prolongation of the developmental cycle duration.

## Conclusion and Perspectives

This study helped us pointing out the effects of biochar from dead leaves of neem and those of mycorrhizae fungus on the larval developmental of cycle of *S. exigua* and the protection of *A. cepa* plants in farm. The relationships between the plants and the phytophagous insects, and entomophagans are very complex and evolve permanently. It clearly appears that secondary substances of plants have a significant role not only in the defence processes but also in their settlement, maintenance and in the evolution of multitrophic relationships; for along with the evolution, all the living things, for their survival, are

constrained to develop a whole panel of defence strategies against their aggressors. In fact, these aggressors, by the development of counter-defensive strategies, are always present. For the maintenance of the biodiversity, a dynamic equilibrium therefore takes place between aggressors and aggressed.

Notwithstanding, there is a need for further research on foliar analysis aiming at understanding and explaining the chemical composition of secondary substances produced by these plants under a synergic action of biochar and mycorrhizal fungi and to spread this technique to the benefits of agricultural undertakers.

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