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Effect of Neem Oil Against Leafminer (*Liriomyza spp*) (Diptera: Agromyzidae) on Field Tomato (*Lycopersicon esculentum* Mill.) in Northern Benin Republic

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***Abstract*— The objective of the study is to increase tomato production by proposing an environmentally friendly alternative. The aim is to test the efficacy of neem oil in the control of leafminer on tomato varieties. The experimental design used is a split-plot with 3 repetitions, with treatments as the main factor (control and neem oil treatment) and varieties as a secondary factor (NADIRA, HEINZ1370 and JAGUAR). The treatments were applied twice during the trial. The frequency of observations on leafminers was 7 days. The study showed that neem oil was significant on *Liriomyza spp* by revealing an average number of 4.21 *Liriomyza spp* with the neem oil treatment and an average number of 9.36 *Liriomyza spp* with the control. This study showed that neem oil has given satisfactory results and may be recommended as an alternative against *Liriomyza spp* infested with tomato. The major advantages of using neem consist not only in its wide availability in the tropics but its different mechanism of action. It can help to reduce selection pressure from conventional insecticides and improve resistance management strategies. its low non-target toxicity reduces hazards to farmers, consumers and the environment. Thus, neem products could be well suited as one of the tools in Integrated Pest Management systems.**

***Keywords*— Neem oil, pests, tomato, Liriomyza spp, leafminer**

I. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important edible and nutritious vegetable crops in Benin. It is an important source of income to those engaged in its production. In Benin, tomato increases the benefit of gardeners and producers and offers employment to thousands of people without jobs and contributes significantly to poverty reduction. Tomato plays a vital role in social, economic and nutritional scheme in Benin. It grows both on a small and commercial large scale as a cash crop by the vegetable growers. Both fresh and processed tomato varieties are popular and economically important vegetable crops. Yet, tomato production in Benin was 331.793 tons (t) in 2013 and it is cultivated on 39,849 hectares (ha), i.e. an average yield of 8.3 t ha^{-1}. This average yield of tomato in Benin is low compared with average yields of 51, 41, 36 and 34 t ha^{-1} in America, Europe, Asia and the entire world, respectively [1]. This is due to the fact that tomato production is highly constrained by several factors [2] including insect pests and disease. Of the most important insect pests that is constraining tomato production is the tomato leafminer - *Liriomyza spp*.

The genus *Liriomyza*, first documented in 1894, contains more than 300 species, with 23 species of economic importance. Larvae can cause severe damage to agricultural and ornamental plants by feeding on mesophyll tissue of leaves and forming serpentine mines [3]. Several species of the genus cause damage to a wide range of crops. On tomatoes, the most important species are the vegetable leafminers, Liriomyza *sativae* Blanchard, and *L. trifolii* (Burgess) [4]. The highly polyphagous *L. sativae* attacks a wide variety of crops and in tomatoes it can cause losses of up to 70% [3], [5].

In previous years leafminers such as *L. sativae* were controlled primarily by the use of synthetic insecticides, such as permethrin and fenvalerate [6]. However, frequent applications resulted in the selection of pesticide-resistant strains, increased health risks to farmers and consumers from persistent residues on produce, and adverse effects on non-target organisms, namely, beneficial parasitoids and predators [7]. Moreover, the classical 'secondary pest' problem can locally increase the leaf miner problem. Long-term use of broad-spectrum pesticides, e.g. methomyl to control primary vegetable pests such as *Helicoverpa zea* (Boddie), *Keiferia lycopersicella* (Walsingham) and *Spodoptera exigua* (Htibner), resulted in outbreaks of *L. sativae* on fresh market tomatoes by destruction of the agromyzid's effective natural enemies [8], [9].

These drawbacks of synthetic pesticides have increased consumers' and growers' interest in natural insecticides originating from plants and their usage has increased in recent years. The advantages of bio-pesticides such as rotenone, pyrethrins and neem include their rapid breakdown in the environment, low human toxicity, lower risk of selecting resistant pest biotypes, and selectivity favoring some beneficial organisms. Numerous studies have shown that applications of azadirachtin can affect many important pests of agricultural and horticultural crops [10], [11] with moderate or low toxicity to nontarget organisms [12], [13]. Residues on plants, plant products or in the soil degrade rapidly, particularly if exposed to UV radiation [14].

Leafminers are sensitive to neem treatments, as shown in studies with *L. trifolii* and *L. sativae* [15], [16], [17]. In addition to larval mortality, sub-lethal effects include both lower fecundity and reduced longevity of adults.

Hence an endeavour has been made to evaluate the efficacy of neem oil as ecofriendly insecticide against tomato leafminer. The main objective is to bring market gardeners, in urban and peri-urban areas of Benin, to the use of botanical insecticides (Biopesticides) as alternatives to chemical insecticides in the control of pests and / or virus vectors of their crops.

II. MATERIAL AND METHODS

1.1 Environmental conditions

The present study was carried out during July to December 2015 at the Experimental Farm of the Faculty of Agronomy of the University of Parakou (FA/UP), located in the Department of Borgou, Regional capital of North Benin, at 09°57'N latitude; 02°43'E longitude and at an altitude of 367 m. The climate is tropical with two seasons, a dry season from November to March and a rainy season from April to October. The recorded annual precipitation varies between 1,000 mm and 1,500 mm for 75 to 140 effective days of rain. The maximum occurs between July, August and September. The lowest temperatures are recorded during the months of December-January. The temperature during the growing season is between 30°C and 31°C. The region stands out pedologically by the predominance of light-textured soils, of significant thickness due to low erosion. As for the vegetation, it is made up of gallery forest and shrub and tree savannas [18].

1.2 Field preparation and experimental design

Three varieties of tomato NADIRA, HEINZ1370 and JAGUAR were purchased from BENIN SEMENCES (Leader in vegetable seeds in Benin, Benin Semences is a limited liability company whose main activity is the sale of vegetable seeds adapted to tropical areas). Neem oil (Azadirachta *indica* A. Juss) was used as a treatment product. Indeed, neem oil, obtained by milling and cold pressing the dry seed, was received from a local producer of neem products (Coobeneem, Parakou, Republic of Benin; 0.009% azadirachtin, analysed by Trifolio-M GmbH, Germany). The treatments are carried out on populations of *Liriomyza spp*. naturally present in the experimental tomato plot.

The experimental design in the study was a split-plot with 3 repetitions, where "treatments" were the first factor and had 2 modalities: control (water treatment) and treatment with neem oil and "varieties" were the second factor and had 3 modalities: NADIRA, HEINZ1370 and JAGUAR. Treatments were placed in the main plots and tomato varieties were placed in the sub-plots. Each subplot had an area of 3 m^2 (3 m x 1 m = 3 m^2) and consists of a total of 18 subplots (2 treatments x 3 tomato varieties x 3 repetitions). Each subplot had 10 plants established in 2 rows of 3 m long, with 0.60 m between rows and 0.50 m between plants on the row. The lane between blocks was 1 m and the subplots were separated from each other by 0.50 m of spaces. The total experimental area covered 54 m^2 (3 m^2 x 18 subplots).

The nursery was carried out at the Experimental Farm of the FA/UP. The manure was mixed with sand in an amount of 50% of each component. The sowing of the three varieties of tomato took place on July 22, 2015. After emergence, the straw was removed and a shady area was put 25 cm above the nursery to protect the plants from sunlight. The watering rhythm was reduced from twice a day at the beginning of the trial to once a day from 15 days after emergence. The shading was removed as the plants developed. The shadehouse was completely removed 5 days before transplanting to gradually acclimatize the plants prior to transplanting. Cleaning and weeding were carried out regularly at the nursery and in the surrounding area to ensure the normal and healthy development of the tomato seedlings. Observations and checks on the health status of the seedlings were noted daily, mentioning any abnormalities due to fungal, viral or insect attacks, and especially the possible presence of tomato leafminer. If the first symptoms of pest attack are observed, the affected plants should be removed and burned.

Planting took place on August 17, 2015, i.e. 26th day after sowing (DAS). After one month of transplanting, hoeing and NPKSB-based fertilizer amendment (14-23-14-5-1) were applied, at 2 kg in two applications, i.e. 1 kg for the first application at 41st DAS and 1 kg for the second application two weeks after the first (55th DAS). Hoeing, ridging and stubble ploughing, which consist of working the soil superficially and minimizing the risk of weed development, were applied whenever necessary. In addition to tillage, regular manual weeding was carried out without the use of herbicides.

1.3 Preparation and Application of the treatment product

The preparation of the treatment product was carried out at the experimental plot. It consisted in adding 45 ml of neem oil to 1.5 L of water. After mixing well, a 1.5 ml emulsifier was added [19]. In the case of our trial, the emulsifier used was the liquid soap commonly used in motorcycle washing services. It is essential to add the soap and mix well. The emulsifier must be used immediately to prevent oil droplets from rising to the surface. Approximately 500 L of this preparation is needed for one hectare, or about 15 L of neem oil. Field treatment with Neem oil was carried out twice during the trial period at the same rate using a hand-held sprayer equipped with a fine mist nozzle. Sprays were made in the evening; 1.5 L of product was sprayed on 9 subplots at a rate of 3 subplots per block. As for the remaining 9 sub-plots, the treatment was done with water. The first treatment was carried out on September 22, 2015 and the second one week after the first one (September 29, 2015).

1.4 Data collection

Observations were carried out at each plot at 7-day intervals and generally began as early as the 58th DAS. They included insect counts (*Liriomyza Spp*.), damage assessment (affected plants and plants having degenerated following pest attacks, as well as plants showing disease symptoms) and determination of yield components. The counting of insects was done (before and after treatment), using a hand magnifying glass, on 5 plants chosen at random in each plot. Adults of *Liriomyza spp*. were counted on the plants since the eggs and larvae of these leafminers were considered too small to be easily counted. After each harvest, the fruits were sorted according to whether they were good or attacked by *Liriomyza spp* and then weighed. Observations and harvests for yield estimation were made on the entire elementary plot. Once the yield per elementary plot was known, the yield per hectare was expressed by extrapolation.

1.5 Statistical analysis

The Excel 2007 software was used for data entry and organization as well as the creation of graphs. The corrected and formatted data totals were analyzed using SPSS (Statistical Package for the Social Sciences) version 22. For the Analysis of Variance (ANOVA), the means were separated by Duncan's test at the 5% threshold.

III. RESULTS

The results of the effect of neem oil on the average number of leafminers (*Liriomyza spp*) per plant in tomato varieties are shown in *Fig. 1* and *2*. Also the distribution of leafminer populations (*Liriomyza spp*) by period, variety and treatment was used to establish *Fig. 1*.

The observation with a magnifying glass of the plants selected at each treatment allowed us to count the population of *Liriomyza* *spp*. This population of *Liriomyza spp* was more raised with the control than with neem oil. The variety NADIRA recorded its abundance peak at 60 days after transplanting (DAT) with an average number of 12.33 *Liriomyza spp* per plant. While the varieties HEINZ1370 and JAGUAR recorded their abundance peaks at 53 DAT with averages of 10.67 and 12.40 *Liriomyza spp* per plant, respectively. During the observation period, the variety JAGUAR presented the highest average number of *Liriomyza spp* per plant (*Fig. 1*).

The results of the statistical analysis in terms of average number of *Liriomyza spp* per plant show that the varieties and the interaction treatments-varieties are not significant, while the treatments are statistically significant (*Fig. 2*). The highest average number of *Liriomyza spp* per plant was obtained from varieties applied to plots containing the water treatment (control) with 9.36 pests while the lowest average number of *Liriomyza spp* per plant was noted in variety applications made on plots containing the neem oil treatment with 4.21 pests. As for the highest average number of leafminers (*Liriomyza spp*) per plant in terms of varieties, it was observed in the variety JAGUAR with 6.96, while the lowest was obtained from the variety NADIRA with 6.87.

Effects of the different treatments according to the varieties on the average mass of healthy fruits, fruits attacked by *Liriomyza spp* and the yield in t ha^{-1} are shown in the Table. Analysis of variance indicates that there is a highly significant difference between treatments but not between the interaction treatment-variety on all components of measured yield however the varieties were only significant on the mass of the attacked fruits. Thus the HEINZ1370 variety was the most exposed to the attacks with an average mass of 884.17 g while the JAGUAR variety was the least attacked with an average of 600 g.

IV. Discussion

The peaks of *Liriomyza spp* abundance were reached at the corresponding period, in each case at the fruiting stage of the plant. These periods of high abundance of leafminer populations could be the result of good plant development. These results disagree with the work of Burban [20] who showed that whitefly populations were high on young leaves and plants at the vegetative stage of development. In fact, *Liriomyza spp* is polyphagous and attacks several cultivated and non-cultivated plants throughout their entire development cycle; the more the plant grows, the more it attracts insects from host plants located in the immediate vicinity of the tomato plot. The plants tested had lush vegetation that attracted many leafminers (*Liriomyza spp*). It was noted that the number of captures increases according to the population dynamics as well as the growth of the plants and especially with the increase in temperature according to the work of Kisserli et al. [21] which mentioned that the higher the temperature, the higher the number of generations. In addition, the results indicate that the application of neem oil reduced the evolution of *Liriomyza spp*. Jotwani and Srivastava [22] showed a significant effect of neem extract on the larval populations of *Heliothis zea* and *Heliothis virescens*, which were declining. Similarly, the leafminer population, which was much higher in the control plots than in the plots treated with neem oil, shows the effectiveness of this product against this insect (*Liriomyza spp*).

Analysis of the results shows that neem oil gives results close to those obtained by Traore et al. [23], who worked on the effect of extracts of Gambia tea and neem on *Helicoverpa armigera* and tomato *thrips*. Its action on leafminers (*Liriomyza spp*) is explained by its growth-inhibiting effect on insects mentioned in the literature [24], [25]. Without killing them immediately, it modifies their growth and prevents them from reproducing. Since they can no longer feed, these pests eventually die. Neem leaves and seeds contain a major substance (azadirachtin) which is effective on soft-bodied insects [26]. The results of the present study corroborate those obtained by Gouba [27] who worked on the effect of neem extract on *Helicoverpa armigera* larvae. The research results obtained by Ekra [28] on okra pests are similar to those obtained in this study. According to Zongo et al. [29], neem extracts control Lepidoptera and Coleoptera populations. Furthermore, our results show a strong influence of neem oil on the average number of leafminers (*Liriomyza spp*). These results are in agreement with those of Jacobson [30] who indicated that the use of extracts of the above-mentioned plant would be an alternative to exclusively chemical control in order to control certain crop pests in Sahelian countries. Ahmed and Grainge [31] also showed that neem grains have an inhibitory effect on larval development and loss of egg fertility with certain Lepidoptera. In the same dynamic, the work of Gnago et al. [32] on pests of okra and cabbage gave conclusive results to the treatment with neem seed extract. However, some researchers have obtained results in contrast to our study. In fact, Hossain and Poehling [33] reported that neem spraying did not influence oviposition by *L. sativae.* In a choice experiment, Webb et al. [15] found no effects on oviposition of *L. sativae* when lima bean plants were sprayed with neem seed kernel extracts (NSKE). Weintraub and Horowitz [34] reported that spraying of 15 ppm Neemix-45 (4.5% azadirachtin) on bean plants *(Phaseolus vulgaris)* had no effect on oviposition of *L. huidobrensis.* Larew [35] reported that a foliar spray with 0.5% NSKE in 0.25% Tween-20 did not inhibit oviposition of *L. trifolii* in bean plants in a greenhouse experiment.

At the level of the susceptibility to attacks, the variety HEINZ1370 was more sensitive and JAGUAR seemed to be less susceptible to attacks. These differences between varieties would result from the expression of the hybrid characteristic, with tolerance to several pests or diseases of certain varieties. Concerning the yield components, the results obtained for the count of healthy fruits, show that the number of healthy fruits produced on the plots treated with neem oil is significantly higher than that obtained in the control plots. This trend can be explained by the fact that neem oil provides protection against the fungi and bacteria that are often responsible for fruit drop. The significantly favorable effect of neem oil on yield is also a convincing result of this study. This appears to be the result of the higher number of fruits produced, relatively higher average fruit weights and low fruit damage rates recorded under neem oil-treated crop conditions. The application of neem oil resulted in the best yields. This shows that the product is necessary to increase tomato yield. Yields of the varieties compared to those obtained by other tomato varieties in the study area were high. The work of Van der Vossen et al. [36] reported tomato yields of less than 15 t/ha in many sub-Saharan African countries. These yields are lower than those recorded in this study. The high performance of these varieties would result from the expression of their hybrid trait, with tolerance to several pests or diseases. Indeed, the performance of tomatoes varied according to agro-ecological factors. The more the biotic and abiotic factors are controlled, the higher the yields obtained by the varieties [37]. Huat [38] confirms these results on the factors that limit tomato yield in northern Senegal. The effectiveness of phytosanitary treatments using the alternative of neem oil would thus play an essential role in tomato production by reducing the level of pest abundance and damage caused by pests.

V. CONCLUSION

The results of our study demonstrate a high efficacy of neem as a foliar application against *Liriomyza spp*. We observed a complete collapse of *L. sativae* populations in successive generations on the field. The major advantages of using neem consist not only in its wide availability in the tropics but its different mechanism of action. It can help to reduce selection pressure from conventional insecticides and improve resistance management strategies. Furthermore, its low non-target toxicity reduces hazards to farmers, consumers (residues) and the environment. Thus, neem products could be well suited as one of the tools in Integrated Pest Management systems. Continuing studies will focus on possible side effects of neem on parasitoids of *Liriomyza spp* on field and on an attempt to work out a strategy of combining parasitoids with neem treatments.

TABLE. *EFFECT OF NEEM OIL ON HEALTHY FRUITS, ATTACKED FRUITS AND YIELD IN TOMATO VARIETIES*

		Treatments		
	Varieties	**Control**	**Neem Oil**	**Mean**
Healthy fruits (g)	NADIRA	3866,67	5923,33	**4895,00**
	HEINZ1370	3571,67	5350,00	**4460,83**
	JAGUAR	3913,33	6766,67	**5340,00**
	Mean	**3783,89b**	**6013,33a****	**4898,61**
Attacked fruits (g)	NADIRA	1110,00	476,67	**793,33ab**
	HEINZ1370	1035,00	733,33	**884,17a***
	JAGUAR	880,00	320,00	**600,00b**

	Mean	**1008,33a****	**510,00b**	**759,17**
Yield ($t\ ha^{-1}$)	NADIRA	16,59	21,33	**18,96**
	HEINZ1370	15,36	20,28	**17,82**
	JAGUAR	15,98	23,62	**19,80**
	Mean	**15,97b**	**21,74a****	**18,86**

Means in the same row or column followed by the same letter are not significantly different according to Duncan's test (P<0.05); (*): significant at 0.05 probability level; (**): significant at 0.01 probability level.

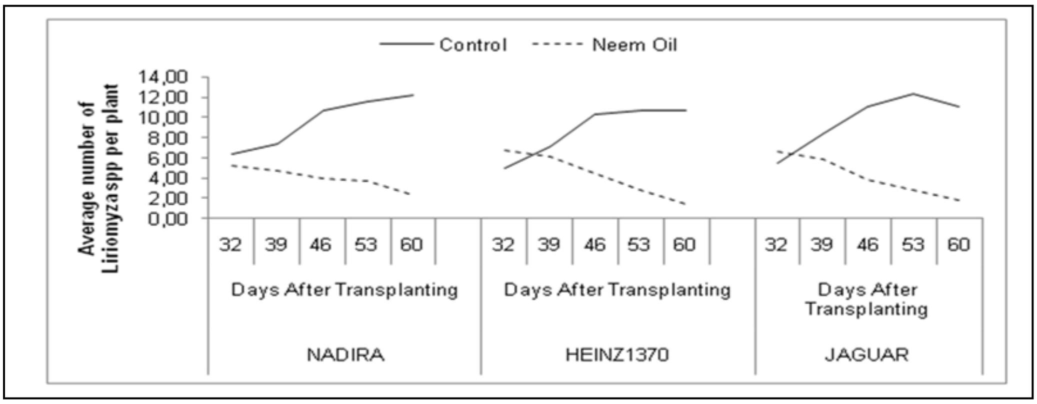

Fig. 1. *Evolutions of the leafminer population (Liriomyza spp) by period, variety and treatment.*

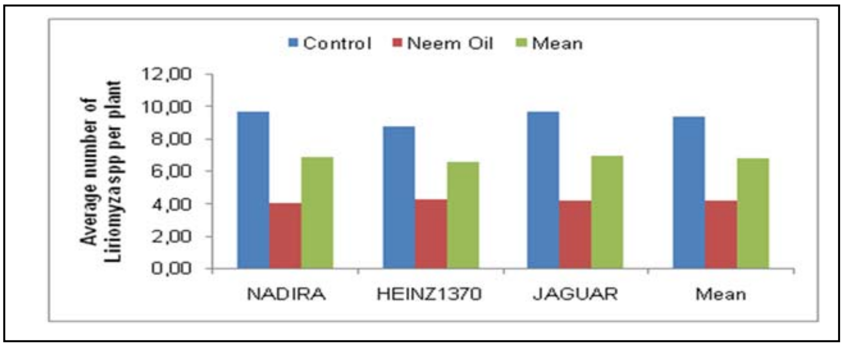

Fig. 2. *Effect of neem oil on the leafminer (Liriomyza spp) in tomato varieties.*

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