International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 7, No. 2, p. 190-198, 2015

INNSPUB

RESEARCH PAPER OPEN ACCESS

Neem seed extract (*Azadirachta indica* A. Juss) (Meliaceae) protects tomato crops (*Lycopersicon esculentum* Miller) (Solanaceae) against T*etranychus urticae* Koch (Acari: Tetranichidae) pests

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Article published on August 27, 2015

Key words: *Tetranychus urticae*, neem seed, acaricide, protect, Tomato.

Abstract

Tetranychus urticae is the main pest rapidly developing resistance to chemical acaricides used to treat tomato (*Lycopersicon esculentum*) in the Far North Region of Cameroon. In an effort to develop an alternative tomato production strategy, a survey was undertaken on 100 producers to collect information on this crop and field experimentation to determine the effectiveness of the powder of neem seeds was performed. The results show that 63.2% of producers recognize tomato pests. Mites are the most important followed by whiteflies. Producers protect their culture with chemical pesticides (70%) or with available products: soap (26%); cow urine (30%). 82% use the neem seed to treat tomato crops. Treatment with neem seed powder shows that it improves the development of tomato ($P > 0.05$) and fruit yield (977 fruit) compared to the control (514 fruit), although it remains below the treatment with chemical acaricide Acarius (1464 fruits). Neem seed powder effectively protects tomato against *T. urticae*.

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Introduction

Tomato, *Lycopersicon esculentum* Miller (Solanaceae), is cultivated under all latitudes, on an area of about 3 million hectares, approximately a third of the world's cultivated land devoted to vegetables (Laterrot & Philouze, 2003; Wahid *et al*., 2014). It is a crop particularly prone to attacks by pests and diseases (Kennedy, 2003). Whiteflies, aphids, leaf miners, mites, thrips, moths and bugs are its main pests (Trottin-Caudal *et al*., 1995). The spider mite, *Tetranychus urticae* Koch, is a major pest of tomato (Gerson *et al*., 2003; Zhang, 2003) which is increasingly becoming highly resistant to the most commonly used acaricides (Mabeya *et al*., 2003; Van Leeuwen *et al*., 2008; Khajehali *et al*, 2009).

The damage caused by Tetranychidae is essentially foliar. The first attacks pass often unperceived, because they are materialized only by some white spots, caused by nutritional bites which destroy the cells of the foliar parenchyma. During severe attacks, these bites cause leaf drop and subsequently a reduction in productivity (Flechtmann & *Knihinicki*, 2002). The accumulation of such damage can even lead to deterioration with death of plants on which these mites develop. Moreover, their webs can grip the organs of the plant and block its development when the density is important (Ferrer, 2009). Thus, at harvest, the losses are estimated at more than 56% in Benin (Azandeme-Hounmalon *et al*., 2014) or even 100% in Cameroon, when the plants are not treated (Fontem *et al*., 1999).

Faced with this situation, farmers use synthetic insecticides to reduce the damage caused by these mites. Among these insecticides, the most used are pyrethroids, organophosphorus or a combination of products (Azandeme-Hounmalon *et al*., 2014; Pahla *et al*., 2014), which are toxic to the users and the environment. Although chemical control is still the most used means today to fight *T. urticae*, it rapidly develops resistance to different families of acaricide molecules (Mabeya *et al*., 2003; Van Leeuwen *et al*., 2008; Khajehali *et al*., 2009) and biological control methods envisaged so far (entomopathogenic fungi, insects and predatory mites) have not led to any effective application (Sarmento *et al*., 2007. ; Aksoy *et al*., 2008; Maniania *et al*., 2008; Biddinger *et al*., 2009; Britto *et al*., 2009). In addition, developed alternative means such as resistant varieties (Rio Grande 1, 2 Rio Grande, Jaguar, Roma VF, Buffalo ...), the use of entomopathogenic fungi and other natural enemies have not led to any effective implementation (Biddinger *et al*., 2009; Britto *et al*., 2009).

Cameroon in general and in the Far North region in particular, mites are the main pests of rural milieu (Fontem *et al*., 1999). More than 70% of losses are observed in the hot and wet season (Benvenuti, 1983). Agricultural performance cannot be achieved without effective crop protection (Britto *et al*., 2009). Protection strategies and stools to fight against these enemies are therefore required to maintain high production. For a long time the extension approach of a method consisted of a technology transfer. However, this approach has been replaced by a participatory approach that involve the producers. Thus, this study aims to reduce mites' attacks through a participatory approach with the involvement of producers in the diagnosis and control through the use of neem seeds as a natural acaricide.

Materiel and methods

Presentation of the study area and the local practice of tomato crop

The Department of Diamaré is located in the Far North region between 10° and 11° north latitude and 14° and 15° East. It is limited to the north by the Department of Logone and Chari, to the west by the Department of Mayo-Sava, east by the Mayo-Danay and south by the Mayo-Kani. Its capital is Maroua. KATCHUNGA, the village where the study took place is about 20 km from Maroua.

The climate in the Department is Sudano-Sahelian type. It is characterized by an average temperature of 27.5 °C, which oscillates between 12-15 C in January and 45 °C in March-April and a very dry atmosphere from March to June. Between October and February, unequal distribution between the duration of the night and the day is observed. The altitude ranges from 300 to 320 meters. (Fotsing, 2009).

A random sample of 100 farmers from five Common Initiative Groups (GICs) of the two Departments of the Region (Mayo-Kani and Diamaré) which are the most important vegetable production basins in the Far North was chosen. These producers were interviewed in order to obtain information on their manner to diagnose cases of attacks of mite and control methods against this crop pest. More specifically, knowledge of pest control methods and timing of intervention, the technical production practices, inputs used and the yields obtained were estimated. If a producer knew all the pests and several control methods at a time, the responses were quantified.

Field tests

Preparation of the test plots and the establishment of culture

The Rio Grande 2 variety provided by SEMAGRI which has a three-month development cycle and gives hard fruit and good color at maturity was chosen. The preparation of the boards and the establishment of the seedbed were conducted according to the principles of the 'Practical Guide to vegetable crops "(Beniest, 1987). Before the establishment of the seedbed, preventive treatment of the plot with SAVANEM at a rate of 20g/4m² was performed seven days after planting, with the aim to fight against nematodes. Insecticide PACHA was used for crop treatment in order to prevent insect attack and ensure that the damage observed is essentially from mites.

Block preparation consisted of deep ploughing and a basal dressing of incorporation (cow manure: 200kg/100m²) and finally levelling and pre-irrigation by runoff. Field transplanting was carried out 25 days after sowing, with two repetitions. The experimental unit (basic plot) consisted of 30 plants with a spacing of 60 cm between rows and 50 cm on the lines and with an area of 10m² (2m x 5m). Weeding and irrigation were performed manually. A maintenance fertilizer (20-10-10, 2kg/l00m²) was made one day after transplanting.

Formulation of acaricides tested and experimentation

Two acaricides in liquid formulations were applied to the plants using a 5 liters ULVA sprayer. The first acaricide is ACARIUS 018 EC based on abamectin and registered in Cameroon as a foliar acaricide under No. 565/10/IN/HOMO/CNHPCAT/CMR is the industrial chemical reference. The second acaricide is an almond solution of neem obtained after collection, drying at 40 °C in the dark in a ventilated room. Steaming was done for 2 days followed by crushing the almonds of neem and sieving to obtain a powder of 0.5 microns in diameter. The liquid formulation of the powder was prepared at a concentration of 18 g/L for the mother solution. For application, a preparation of 1L of almond stock solution was diluted in 4 L of tap water, needed to treat one hectare of tomato plants.

To evaluate the liquid formulations prepared at the Katchunga site, an experimental design in completely randomized blocks was set up. Three treatments were carried out on three plots: A: untreated plot (control); B: plot treated with almond powder of neem; C: plot treated with the reference acaricide ACARIUS 018 EC. The treatments took place every 15 days until harvest.

Observations of ongoing culture

Observations of mite dynamics

20 days after sowing, observations were made every 10 days to track the emergence and evolution of larval populations of mites on plants. The plants presenting signs of attacks were collected and dissected for enumeration of larvae under a magnifying glass. 30 plants in total (15 per plot) are thus examined for dissection and enumeration of larvae.

Mite attacks were recorded regularly; they were reflected in leaf yellowing by convergent beaches and a reduction and an embossing of leaves taking bronze coloration on the lower surface. Any plant showing these symptoms on only one of its leaves was considered as attacked. At each sampling date, the number of affected plants was determined for the whole culture. The evolution of the number of attacked plants during the culture, the sensitivity threshold as well as the characteristics of vegetative development and production were measured.

Estimated impact of the attacks on growth and productivity

In order to establish the relationship between the degree of mites' attack and various treatments, the behavior of plants and their vegetative development and production were evaluated at different treatments. Using a scale of 1 m, height was measured on five plants of each basic unit for each treatment. For this, one of the highest ramifications was considered. To congestion, the longer opposite side branches were measured by a ruler also.

Qualitative observations (shapes, colors, size uniformity) (Gaspari, 2014) were made on all fruits of the first three harvests for each treatment. Yield components (number of fruits, average weight of fruits) of the different treatments were measured on three successive harvests. For average weight of the fruit, a Kern precision balance (1/1000g) was used.

Statistical analysis

The collected data were processed and analyzed with the XLSTAT software Version 2013. This software has permitted a variance analysis to compare the results obtained from the different treatments.

Results

The tomato production system in the Far North of Cameroon

Tomatoes production in the Far North Region is relatively recent; it is practiced only since a little more than one decade. Only 22% of investigated producers have been cultivating tomatoes for more than 12 years while 23% producers have cropping experience under 5 years (Table 1). Producers who have an experience of more than five years are more numerous (77%). However, 47% of producers have practiced this activity for more than 10 years.

Table 1. Numerical importance of the tomato producers according to their seniority.

	Seniority in tomatoes production (Year)		
	2 to 5	6 to 9	More than 10
Producer (%)	23	30	47

Knowledge of tomato pests by investigated producers

Most producers are in general non-specialists in plant protection. With the training received heads of agricultural stations and extension agents have the ability to identify the damage of the main pests of tomato. Based on this knowledge, the various pests that producers recognize as a drawback on tomato production in five production areas of the Far North region of Cameroon in order of importance are presented in Table 2.

Table 2. Level of knowledge of tomatoes main pests by the producers.

	Locality (%)				Average (%)
Pests	Katchunga	Godola	Meskine	Doumrou	
Nematodes	36	40	56	32	41±10,52
Aphids	52	60	44	48	51±6,83
Mites	80	72	88	76	79±6,83
Thrips	72	68	80	72	73±5,03
White flies	76	64	84	68	73±8,87

It is clear from this table that each farmer knows more than a pest and these pests differ relatively from one farm to another. Mites are most prominent in the study area, known by 79% of producers, followed by white flies (73%) and trips (72%).

It is found that over 80% of the producers are victims of mite attacks especially at Meskine compared to nematodes which less than 20% of producers deplore the damage caused by this type of pest.

Table 3. Products to fight against tomatoes pests used by producers.

	% par localité				Average (%)
Product used	Katchunga	Godola	Meskine	Doumrou	
Soap	28	24	20	32	26±5,16
Neem almond powder	88	80	84	76	82±5,16
cow urine	24	32	28	36	30±5,16
Pesticide	60	68	80	72	70±8,33

Control methods used

From the answers of interbiewed producers, it appears that each of them knows more than one control method. Table 3 below highlights the different natural products used by farmers to control the population of tomato pests.

Four types of agronomic solutions are used by the farmers for phytosanitary treatments of tomato and are mobilized according to the nature of the target pest. The investigation revealed that 70% of producers use pesticides to fight against insects; only 26% use soap; 30% spray their culture with cow urine and 82% of all interviewed producers apply the neem almond powder.

Table 4. Characteristic of the production of consumable fruits according to the treatment.

	Number of fruits for 1/4 ha		
Treatments	Total	Consumables	AWF (g)
Any treatment	610	514	45,03 ± 4,98
Neem almond powder	1221	977	51,00 ± 7,38
Acarius 180 EC	1648	1464	60,10 ± 3,04

AWF : Average weight of a fruit.

Experimentation field

Dynamics of mite larvae in the field

In order to follow the evolution of larval populations of mites in the plants, regular sampling of attacked plants (20, 30, 40, 50 and 60 days after sowing) were performed on the control plots. 30 stems in total (15 per plot) were examined for dissection and larvae numeration (Figure 1).

This method help to identify a clear succession of different development stages of mite pests. A young larvae density is noted particularly high at 30 days after transplanting. At 40 days after sowing, pupae are present. No significant difference at the 5% level ($P < 0.03$) appears between 40, 50, 60 days of observation.

Average density of the mite population following the three treatments

The observation of the density of mite population with respect to the various treatments showed significant difference ($p > 0.006$) between treatment A and others. On the order hand no significant difference was observed in the treated samples.

It is clear from Figure 2 that the deterioration is more important and earlier for plants having received no acaricide treatment, while it is limited and late for plots treated with chemical acaricide after 81 days for all observations. There is no significant difference between treatments with neem seed and acarius at the 5% Fisher level ($p < 0.05$).

Vegetative development

Figure 3 shows the vegetative development of tomatoe plants following different treatment types.

It appears from this Figure 3 that there is no significant difference ($P > 0.200$) between the change of the height and size of the plants in the three treatment programs. And this regardless of the presence of mites and the presence of certain disease symptoms. This height varies from 65 to 70 cm depending on the treatment. The congestion is located around 60 cm.

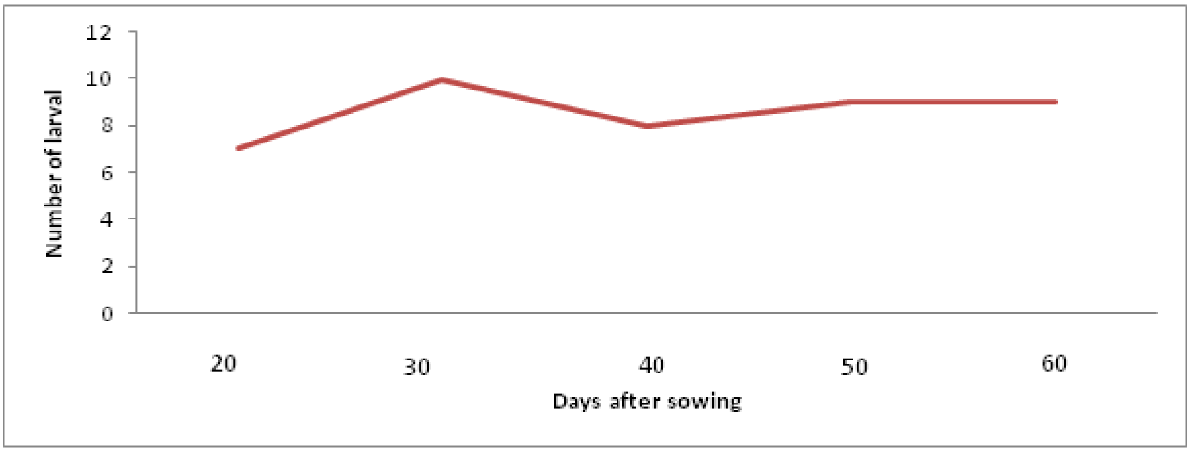

Fig. 1. Evolution of the larval population of mites in the control plot.

Production characteristics

For this study, is considered as a consumable product, one that has a round shape with a uniform size of between 35-45 mm, possessing a bright red color, spotless and having a weight of 40 to 80 g.

Table 4 shows the performance of consumable tomato fruits from different treatments. It is clear from the table that the comparison of the yields obtained by a culture protected by neem seed powder and the active ingredient Abamectin revealed a significant difference ($P > 0.05$) in terms of the average weight of fruits obtained, supplies ranging from 45-60 g. This weight obtained with Acarius treatment is considerably different from other treatments, followed by treatment with neem seed with consumable fruit yields respectively 88.83 and 80.01% of consumable fruit / quarter ha (Table 4).

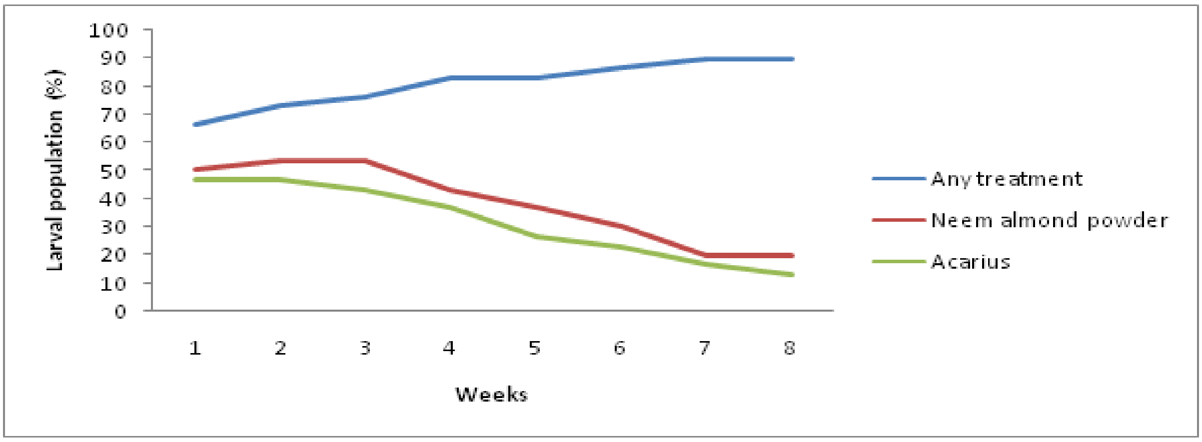

Fig. 2. Percentage of the larval population of mites according to the treatments.

Discussion

The characterization of the studied samples suggests the emergence of tomato crop in the study area. However, farming systems despite their age are described as extensive and strongly consuming the natural resources as noted by some authors in their work (INERA, 2000). The observation made in the study area shows that almost all producers have some knowledge of the various pests in their tomato crop. This attitude can be explained by the synergy between producers and accompaniment services including them in decision making. Use of chemical acaricide is minimal. However several studies have shown the importance of the contribution of a chemical acaricide with strong remanence in the control of mite population (Blair, 1989). However, this situation may progress over time and lead to changes in production systems with an inevitable increase in yields from the producers. The use of organic manure is not widespread among producers while a FAO study (2007) showed that the degradation of pesticides is higher in soils amended with organic matter compared to non-amended soils. This could explain the very rapid development of resistance mechanisms to chemical acaricide by weaver mites.

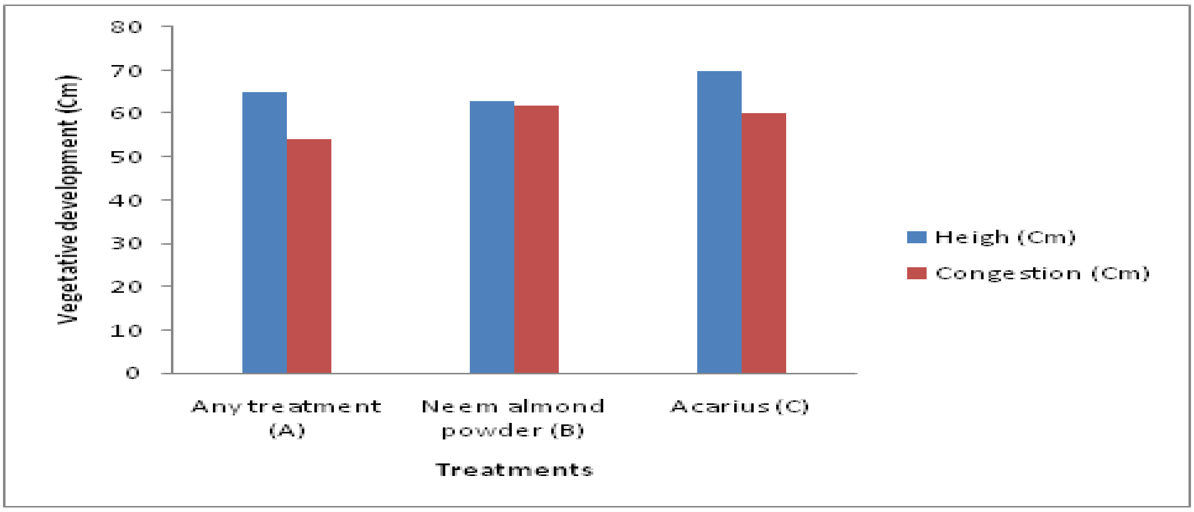

Fig. 3. Vegetative development of the plants according to the treatments.

Significant losses observed on treatment A is largely explained by violent attacks of *T. urticae* on young tomatoes, causing plant death (loss of productive plants / ha) because it is at three to four leaves stage that larval mite population is abundant. It was during this period (30 days) that most of egg laying happens. If no treatment is envisaged on this date, it would be very difficult to achieve control pupae from 40 days of infection (Sougnabé *et al.*, 2009).

The number of infected plants varied depending on the treatment. It could be a rapid loss of effectiveness of the almond powder of neem because this effectiveness is felt at the time of applying, meanwhile the risk of scrubbing is high. It is what explains the decrease in the mite population at treatment period. Following the exceptional climatic conditions of May during which the outside temperature exceeded regularly 30 °C, the multiplication of *T. urticae* was very fast, and required several additional contributions of an acaricide which were distributed uniformly in the compartment C from which the low level of infestation is observed.

A significant difference is also apparent in regard to the vegetative development of the plants ($p > 0.05$). It should however be noted that the harvesting of large fruits was significant and earlier obtain in the spot treated with a chemical acaricide. According to Stacey (1983), an effective loss of 25% of the leaf surface tomato plants caused by mite bites can be tolerated without loss of yield. The same author shows that despite an artificial defoliation of 3/4 of the surface of each leaf, the tomato plant is still capable of producing 60% of the performance of an entire plant. So there is a compensation mechanism probably related to increased photosynthetic activity of the remaining surface.

The effectiveness of the neem seed powder is due to its various compounds which are concentrated in grains (Singh *et al.*, 2010 cit. Tamgno and Ngamo Tinkeu, 2014). The high toxicity of neem seed powder is due to the high concentration of insecticidal, repellents, antifeeding, phagodissuasifs or growth regulator compounds in those parts of the neem (Tamgno and Ngamo Tinkeu, 2014). Its seeds are used to make a dangerous insecticide, theazadirachtin which is biodegradable, creates no resistance in insects and is very economical (Berger, 2011).

Conclusion

Applications of different doses of neem seed powder have a significant effect on the vegetative development and production of tomato. The chemical method using ACARIUS was more effective than neem almond powder in production rates. Producers can reduce the processing frequency of 15 to 10 days to have a good efficiency of this powder. Thus, the high level of biological activity of the neem seed suggests that it could be used as a base substrate for the formulation of insecticides (acaricides) to be used an an alternative against tomato mites in the field. The results of this study are of high importance for small producers of tomatoes that are not always able to master the techniques involved in mining operations and may have difficulty accessing commercial products. Given current concerns, the use of formulations of neem seed can be an interesting and promising alternative for the Cameroonian agriculture.

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