Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Characteristics of tomato plants treated with leaf extracts of neem (*Azadirachta indica* A. Juss. (L.)) and mata-raton (*Gliricidia sepium* (Jacquin)): A greenhouse experiment

Joaquín Adolfo Montes-Molina[1], Ibis Harumy Nuricumbo-Zarate[1], Javier Hernández-Díaz[1], Federico Antonio Gutiérrez-Miceli[1], Luc Dendooven[2] and Víctor Manuel Ruíz-Valdiviezo[1]*

[1]Laboratory of Biotechnology, Instituto Tecnológico de Tuxtla-Gutiérrez, Tuxtla-Gutiérrez, México. Carretera Panamericana Km 1080, Tuxtla Gutiérrez, Chiapas

[2]Laboratory of Soil Ecology, Cinvestav, Av. I.P.N. 2508 C.P. 07360, México D. F., México

*Corresponding Author E-mail: bioqvic@hotmail.com

Publication Info

Paper received:
14 June 2013

Revised received:
22 December 2013

Accepted:
08 March 2014

Abstract

Extracts of neem (*Azadirachta indica* A.) and mata-raton (*Gliricidia sepium*) leaves were used as insect repellent during organic cultivation of tomato plants (*Solanum lycopersicum*) and were compared with untreated plants or plants treated with lambda-cyhalothrin (chemical treatment). The best developed tomato plants were found in the Gliricidia treatment, while difference between other treatments were small. The number of different species of macrofauna found on tomato plants were similar in different treatments, except for corn rootworm (*Diabrotica* spp.) found in the Gliricidia treatment, but not in other treatments. It was found that leaf extract of *G. sepium* stimulated tomato growth and altered the leaf and fruit characteristics. This was most likely due to its action as a growth regulator and/or an inductor of changes in the tomato growth regulation, but not due to its action as an insect repellent. Consequently, leaf extract of *G. sepium* could be used to stimulate tomato development.

Key words

Azadirachta indica, Bioinsecticides, *Diabrotica* spp., *Gliricidia sepium*

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), tomato (*Solanum lycopersicum* L.) is one of the most cultivated plant in the world with a production of approximately 145,000,000 tons in 2010 (http://faostat.fao.org). In the state of Chiapas (Mexico), tomatoes are mostly cultivated organically, but production is often low due to diseases favoured by climatic conditions, such as high humidity and temperatures. The incidence and damage by pathogens vary between seasons, regions and the year, but losses can be large (Villar and López, 2003).

Of the many insects that feed on tomato plant, the most important is whitefly, *Bemisia tabaci* (Gennadius, 1889). While feeding on tomato plants, whitefly infects them with viruses, e.g. begomoviruses (family Geminiviridae, genus *Begomovirus*), which often limits the cultivation of crops (Diaz-Pendon *et al.*, 2010). Cover cropping with polypropylene mesh has been introduced to avoid contact between the plant and whitefly in the early stages of development, resulting in high yields with good fruit quality. Corn root worm *Diabrotica* spp. (Chevrolat in Dejean, 1836) is another pest that feeds on tomato plants (Pedersen and Godfrey, 2011). The larvae develop in soil feeding on roots, while the adults eat leaves, flowers, buds and pods, damaging the plant seriously.

Damage to crops by insects is highly variable in Chiapas, but can be as high as 90% (Montes-Molina *et al.*, 2008). Traditionally, chemical insecticides, such as lambda-cyhalothrin, are used to control pests on crops. However, chemical insecticides might affect the environment and kill predators that prey on insects that cause damage to a crop. Lately, extracts of plants have been used to control insects that reduce crop yields. Plant extracts have the advantage that they can be obtained locally at a low cost and it is hoped that these, when applied have a less adverse effect on ecosystem. Of these, seeds or extracts of neem plant (*Azadirachta indica*), leaf are widely used and have proven to be effective pesticides (Salako *et al.*, 2008). Neem belongs to family Meliacea and has been studied widely for its use as a bio-insecticide (El Shafie and Basedow, 2003). Neem extracts, with more than 300 identified components, alters the behaviour and physiology of mites, insects and nematodes (Schaaf *et al.,* 2000). Azadirachtin A is the main insecticidal ingredient in neem plant (Boursier *et al.*, 2011). Neem is a native plant of India and it is not known how cultivating it might affect ecosystems in Southern Mexico. *Gliricidia sepium* (Jacquin) belongs to family Fabaceae and originates from Central America. It is a leguminous tree used as live fences and sometimes as fodder. Extracts of *Gliricidia sepium* (Jacquin)*,* a plant locally used as repellent, has been less used as bioinsecticide. Larvicida activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephensi* (Liston, 1901), *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823) has been reported by Sharma *et al.* (1998). In previous experiments, it was found that leaf extracts of *G. sepium* reduced damage to maize plants and increased yields, although to a lesser extent than when leaf extracts of neem were used (Montes-Molina *et al.*, 2008).

As part of a study into the use of plant extracts as insect repellent, in organic farming, leaf extracts of neem and *G. sepium* were applied to tomato plants cultivated in a greenhouse. Plant development, fruit characteristics, and macrofauna on tomato plants were monitored in the greenhouse. The objective of this study was to determine how leaf extracts of neem or *G. sepium* affected the macrofauna of tomato plants cultivated in greenhouse, growth of tomato plants and characteristics of tomato fruits. This is the first report on the use of leaf extracts of both neem and *G. sepium* as bio-insecticide in the cultivation of tomato plants.

Materials and Methods

Leaf extract preparation : Twenty mature neem (*A. indica*) and 20 mata-raton *(G. sepium)* trees were selected random by at the South-East of Tuxtla Gutierrez. Five hundred grams leaves were sampled from each tree and pooled. As such, 10 kg leaves were collected. Leaves were at least 3 month old and had lost their flexibility so that insects like ants, could not damage them any more. One hundred grams of fresh leaves were washed with water to clean them, cut in to 2 mm^2 squares, added to 1 dm^3 water, left in dark for 72 hr, which was sufficient to extract most of the active components of neem and mata-raton. The solution was then filtered and made up to 3 dm^3 with water. Extraction procedures were kept as simple as possible to be easily applicable for local farmers. Lambda-cyhalothrin, which served as a chemical insecticide, was obtained from Syngenta (Willmington, Del., USA).

Soil sampling site and characteristics of soil : Soil was collected at the experimental fields of '*Instituto Tecnológico de Tuxtla Gutierrez'* in Tuxtla Gutierrez. Its average altitude is 570 m above sea level and characterized by a hot sub-humid climate with mean temperature ranging from 26 to 28 °C and average annual precipitation of 1000 mm mainly from June through August (http://www.inegi.gob.mx). The soil was previously not cultivated for > 30 y and was covered with grasses and shrubs when the area was prepared for sampling. The soil, with pH 6.6 and water-holding capacity of 380 g kg^{-1}, had an organic C content of 56.8 g kg^{-1}, an inorganic C content of 2.8 g kg^{-1} and total N content of 8.9 g kg^{-1} soil. The particle size distribution of the soil was 50 g clay kg^{-1} (< 0.002 mm), 50 g silt kg^{-1} (> 0.002 mm and < 0.05 mm) and 900 g sand kg^{-1} (> 0.05 and < 2 mm).

Cultivation of tomato plant and application of the leaf extracts of neem and *Gliricidia*: The experiment was done in a greenhouse from April to July in 2007, 2008 and 2009. The daily temperature during the experimental period ranged from 24 to 36 °C. The plants were planted in plastic containers (diameter 30 cm and 40 cm depth) containing 10 kg soil on a dry weight base and placed in a greenhouse. Each third day, 2 dm^3 tap water was added to each container. Thirty-six plastic containers were used for each of the five treatments. As the experiment was repeated three times to account for climatic differences, a total of 108 plants were used for each treatment.

Tomato plantlets were kept ten days under an anti-aphid net and then left in the open for ten days so that insects could infect the plantlets. After ten days, the plantlets were placed under an anti-aphid net again, two plantlets were discarded and four different treatments applied. In a first treatment, the two plantlets cultivated in each recipient were sprayed with 5 ml extract of *Gliricidia* leaves (considered the Gliricidia treatment), in the second treatment the two plantlets cultivated in each recipient were sprayed with 5 ml extract of neem leaves (considered the NEEM treatment), in the third treatment the two plantlets cultivated in each recipient were sprayed with 5 ml lambda-cyhalothrin solution (considered the chemical treatment). The amount of lambda-cyhalothrin sprayed onto the plantlets was similar to the amount recommended during the cultivation of tomatoes. The procedure of spraying 5 ml leaf extracts or lambda-cyhalothrin on the plantlets was repeated every week until six sprayings were applied. In a fourth treatment, no insecticide was applied to the plantlets (considered the control treatment). In the experiment, a plant density of approximately 60000 plants ha^{-1} was obtained, which is the recommended density for tomatoes (INIFAP-CECECH, 1999).

The plants were fertilized with urea for a total of 150 kg N ha^{-1}, i.e. 75 kg at 15 days after emergence and 75 kg at 45 days.

Triple 17 (17% N, 17% P as P_2O_5 and 17% K as K_2O) was applied at 15 days after emergence. The dose applied considered that 60,000 plants were cultivated per ha. The plantlets were further cultivated under the anti-aphid net until harvested. The time of flowering and the number of flowers was determined. At harvest the fruits were counted, weighted, dried in the shade for two weeks and weighted again. After harvest, the soil was washed from the roots, the roots were removed from the above ground plant material and weighted. The roots and above ground plant material was dried in the shade for two weeks and weighted again. The leaves were removed from the stem and hammer milled < 0.1 mm prior to characterization.

Bromatological characteristics of the plants: The characteristics were determined on fresh fruits and dried tomato leaves. The water content, acid and fibre content, total N, pH, and the % Brix of the leaves and fruits were determined as described by AOAC (1980). The carotenoids and lycopene content was determined as described by Wang *et al.* (2005).

Determination of the macrofauna on the tomato plants: Insects were monitored just before and after fumigation. Flying insects were captured with a 40 cm diameter fine-meshed net, identified and counted while non-flying insects were hand collected, identified and counted (Ortega, 1987). Captured insects were liberated immediately after identification so that changes in the fauna were due to the treatment and not due to the collection. Insect abundance is expressed as the number found. The family and the order to which the insects belonged, their common name, the effect they have on the plants (causing damage) or their action as possible predator (beneficial) is given in Table. 1.

Statistical analysis: Significant difference for plant and fruit characteristics and number of insects as a result of the different treatments were determined by analysis of variance (ANOVA) (PROC GLM, SAS Institute, 1989). The minimum significant difference (P<0.05) was calculated using the general linear model procedure (PROC GLM). This procedure can be used for an analysis of variance (ANOVA) for unbalanced data, i.e. when some data are missing. The number of insects were first log transformed (Log(x+1)) prior the statistical analysis. Data were checked for normality and log (x+1) transformed to reduce skewness and comply with the assumption of homoscedasticity (Webster, 2001).

The relationships between the characteristics of the plant, fruits and number of insects were visualized by principal component analysis (PCA). For fruit and plant characteristics only those that were significantly different between the treatments were retained, while all insects determined before fumigation were considered. Only principal components with Eigenvalues > 1 and explaining > 10% of the total variance were retained. Details of the PCA technique can be found in Valdez-Perez *et al.* (2011).

Results and Discussion

Fresh (total plant, roots and above ground plant material) dry and foliar weight were significantly affected by the treatment (P < 0.05) (Table 2). The best developed plants were found in the Gliricidia treatment, while differences between other treatments were not significant. A higher fruit weight was found in the chemical and Gliricidia treatments as compared to other treatments. These differences were visualized using PCA analysis (Fig. 1a). The Gliricidia treatment was clearly separated from the other treatments. It was characterized by a positive PC1, *i.e.* better developed plants, while differences between other treatments were small.

Table 1 : Insects found on tomato plants (*Solanum lycopersicum* L.) amended with leaf extracts of neem and mata-raton cultivated in the greenhouse

Insect	Common name	Order	Family	Effect[a]
Bemisia tabaci (Gennadius, 1889)	Sweet potato whitefly	Hemiptera	Aleyrodidae	-
Cactoblastis cactorum (Berg, 1885)	Cactus or nopal moth	Lepidoptera	Phycitidae	-
Danaus plexippus (Linnaeus, 1758)	Owl butterfly	Lepidoptera	Nymphalidae	-
Diabrotica spp. (Chevrolat in Dejean, 1836)	Corn rootworm	Coleoptera	Chrysomelidae	-
Frankliniella occidentalis (Pergande, 1895)	Western flower trips	Thysanoptera	Thripidae	-
Heliothis spp. (Ochsenheimer, 1816)	Fruit borer	Lepidoptera	Noctuidae	-
Cercopoidea (Leach 1985)	Froghoppers	Hemiptera	Cercopidae	-
Lyssomanes spp. (Hentz, 1845), Pseudicius spp. (Simon, 1885), *Mimetus* spp. (Hentz, 1832), *Peucetia viridans* (Hentz, 1832)	Jumping spiders	Araneae	Salticidae	+
Mantis religiosa (Linnaeus, 1758)	European mantis	Mantodea	Mantidae	+
Messor barbarus (Linnaeus, 1767)	Ant	Hymenoptera	Formicidae	+
Pseudococcus spp. (Westwood, 1840)	Mealy bugs	Hemiptera	Pseudococcidea	-
Sphenarium spp. (Charpentier, 1842)	Grasshopper	Orthoptera	Acrididae	-
Vespa spp. (Linnaeus, 1758)	Yellow jacket	Hymenoptera	Vespidae	-

[a]Effect: when the effect is (-) then the animal is considered a pest and might cause damage to the plant, when the effect is (+) then the animal is considered a predator and might benefit plant growth

Most leaf characteristics were affected significantly by treatment, except for total N, protein, lycopene and carotenoids content ($P < 0.05$) (Table 3). Ash and fibre content was significantly larger in the Gliricidia treatment than in other treatments, but the °Brix, water content and pH were significantly lower than in most treatments.

Consequently, the treatments were clearly separated in the PCA analysis (Fig. 1b). On the one hand, the Gliricidia treatment was characterized by a negative PC1, *i.e.*, a high ash content, and a negative PC2, *i.e.*, high fibre content but a low pH. On the other hand, the other three treatments had a positive PC2 and a small negative or positive PC1. All measured fruit characteristics were affected significantly by treatment ($P < 0.05$) (Table 4). In general, fruit characteristics such as ash, fibre, total N, protein, acid, lycopene and carotenoid content, were larger in Gliricidia treatment and lower in control treatment. Water and sugar content, however, was lower in Gliricidia treatment than in other treatments.

Differences in PC1 value, e.g. sugar content, for different treatments were small (Fig. 2a). However, PC2 factors showed larger differences among treatments. The Gliricidia treatment was characterized by a large positive PC2, *i.e.* a high fibre content and a low water content, that separated it clearly from other treatments.

The number of different species found on tomato plants was similar for different treatments (Table 1). Corn rootworm was found only in Gliricidia treatment, but not in other treatments. Consequently, PCA analysis did not separate the treatments well (Fig. 2b).

Table 2 : The effect of leaf extracts of neem and mata-raton on characteristics of tomato plants cultivated in the greenhouse

Characteristics	Control[a]	Gliricidia[b]	Neem[c]	Chemical[d]	MSD[e]	F value	P value
Fresh plant weight (g)	27[ab f]	48[a]	26[b]	23[b]	21	4.25	0.0073
Fresh foliar weight (g)	24[b]	44[a]	23[b]	20[b]	19	4.43	0.0058
Fresh root weight (g)	3.1[a]	4.0[a]	2.7[a]	2.9[a]	1.7	1.48	0.2252
Dry plant weight (g)	12.8[a]	21.5[a]	12.6[a]	12.0[a]	11.0	2.42	0.0706
Dry foliar weight (g)	10.3[a]	19.1[a]	11.5[a]	10.5[a]	10.1	2.50	0.0640
Dry root weight (g)	1.5[a]	2.1[a]	1.5[a]	1.5[a]	1.1	1.06	0.3685
Plant height (cm)	76[a]	80[a]	70[a]	81[a]	12.2	0.95	0.4203
Foliar height (cm)	59[a]	63[a]	56[a]	63[a]	5	0.36	0.7825
Root length (cm)	16[a]	16[a]	14[a]	16[a]	12	1.88	0.1380
Stem diameter (cm)	1.9[a]	2.5[a]	1.8[a]	2.1[a]	18	0.47	0.7037
Water content (g kg^{-1})	580[a]	510[a]	430[a]	510[a]	220	0.79	0.5042
Flowering (days)	52[a]	51[a]	49[a]	50[a]	7	0.67	0.5709
Fruit forming (days)	53[a]	57[a]	53[a]	57[a]	14	0.43	0.7335
Number of flowers	9[a]	12[a]	11[a]	11[a]	7	0.65	0.5841
Fruit weight (g)	43[a]	48[a]	45[a]	42[a]	17	0.33	0.8048
Fruit diameter (cm)	4.1[a]	4.4[ab]	4.5[a]	4.7[a]	0.7	1.20	0.3147
Fruit length (cm)	4.5[a]	4.8[a]	4.7[a]	4.2[a]	1.0	0.94	0.4267

[a]Control : Plants that were exposed to insects but received no treatment, b Gliricidia: Plants treated with extracts of leaves of *Gliricidia*, c Neem: Plants treated with extracts of neem leaves, d Chemical : Plants treated with Lambda-cyhalothrin, e MSD: minimum significant difference ($P < 0.05$), f values with the same letter within the row are not significantly different between the treatments

Table 3 : The effect of leaf extracts of neem and mata-raton on leaf characteristics of the tomato plant cultivated in greenhouse

Characteristics	Control[a]	Gliricidia[b]	Neem[c]	Chemical[d]	MSD[e]	F value	P value
Ash content (g kg^{-1})	121[b f]	137[a]	114[b]	118[b]	14	9.49	<0.0001
Fibre content (g kg^{-1})	175[c]	218[a]	188[bc]	190[b]	14	24.11	<0.0001
Total N (g kg^{-1})	21[a]	25[a]	22[a]	20[a]	5	2.59	0.0565
oBrix	1.3[bc]	1.1[c]	1.7[a]	1.4[b]	0.2	16.41	<0.0001
pH	7.3[ab]	7.1[b]	7.6[a]	7.7[a]	0.3	8.35	<0.0001
Lycopene (mg kg^{-1})	17.9[a]	14.0[a]	14.5[a]	18.1[a]	6.6	1.75	0.1610
Carotenoids (mg kg^{-1})	67.1[ab]	59.8[b]	63.2[ab]	70.1[a]	9.7	2.94	0.0364
Water content (g kg^{-1})	650[a]	590[b]	640[a]	640[a]	10	70.65	<0.0001

[a]Control: Plants that were exposed to insects but received no treatment, [b]Gliricidia: Plants treated with extracts of leaves of *Gliricidia*, [c]Neem: Plants treated with extracts of neem leaves, [d]Chemical : Plants treated with Lambda-cyhalothrin, [e]MSD: minimum significant difference ($P < 0.05$), f values with the same letter within the row are not significantly different between the treatments

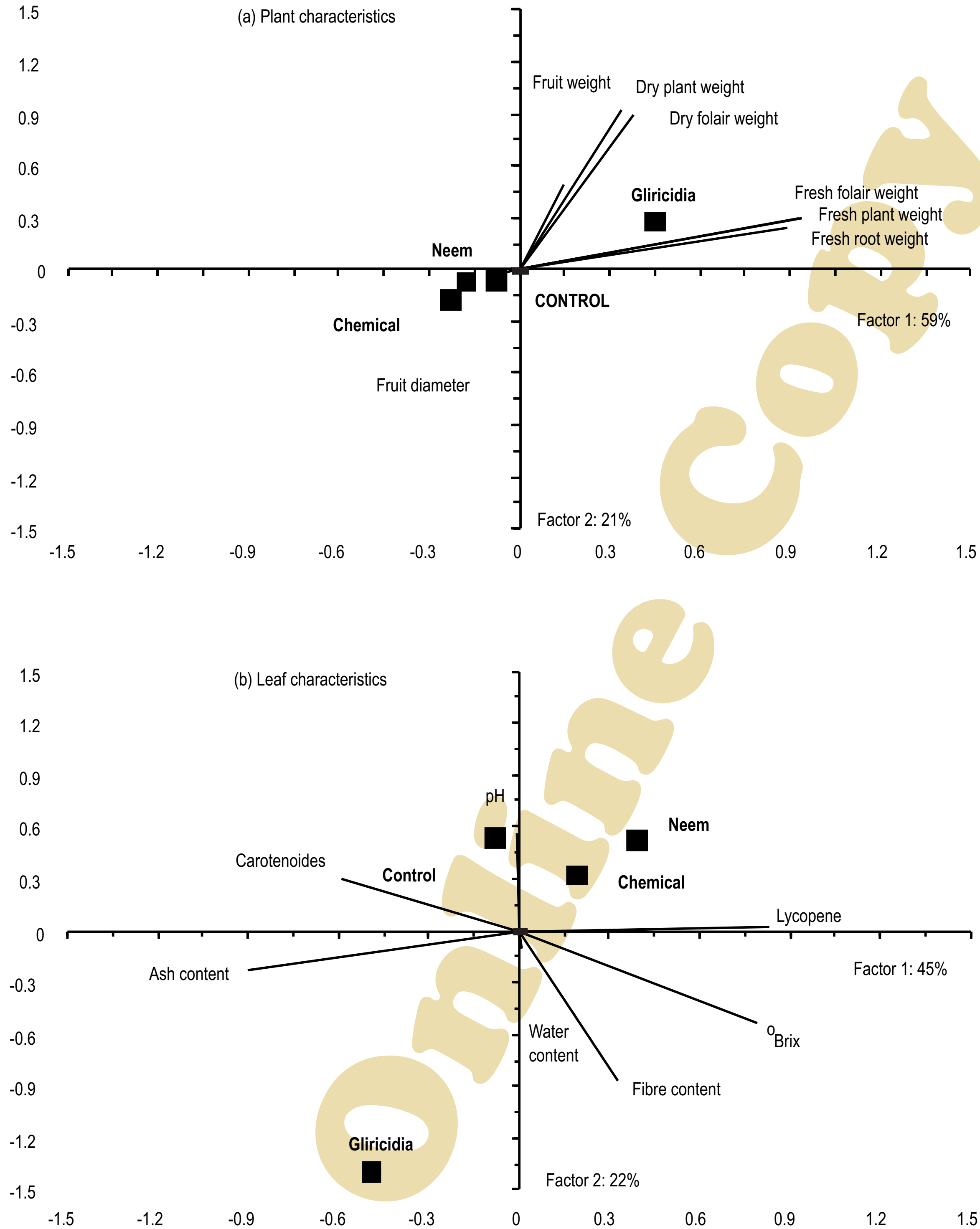

Fig. 1 : Principal component analysis of (a) tomato plant characteristics; (b) leaf characteristics of plants treated with lambda-cyhalothrin, leaf extracts of neem, mata-raton or left untreated

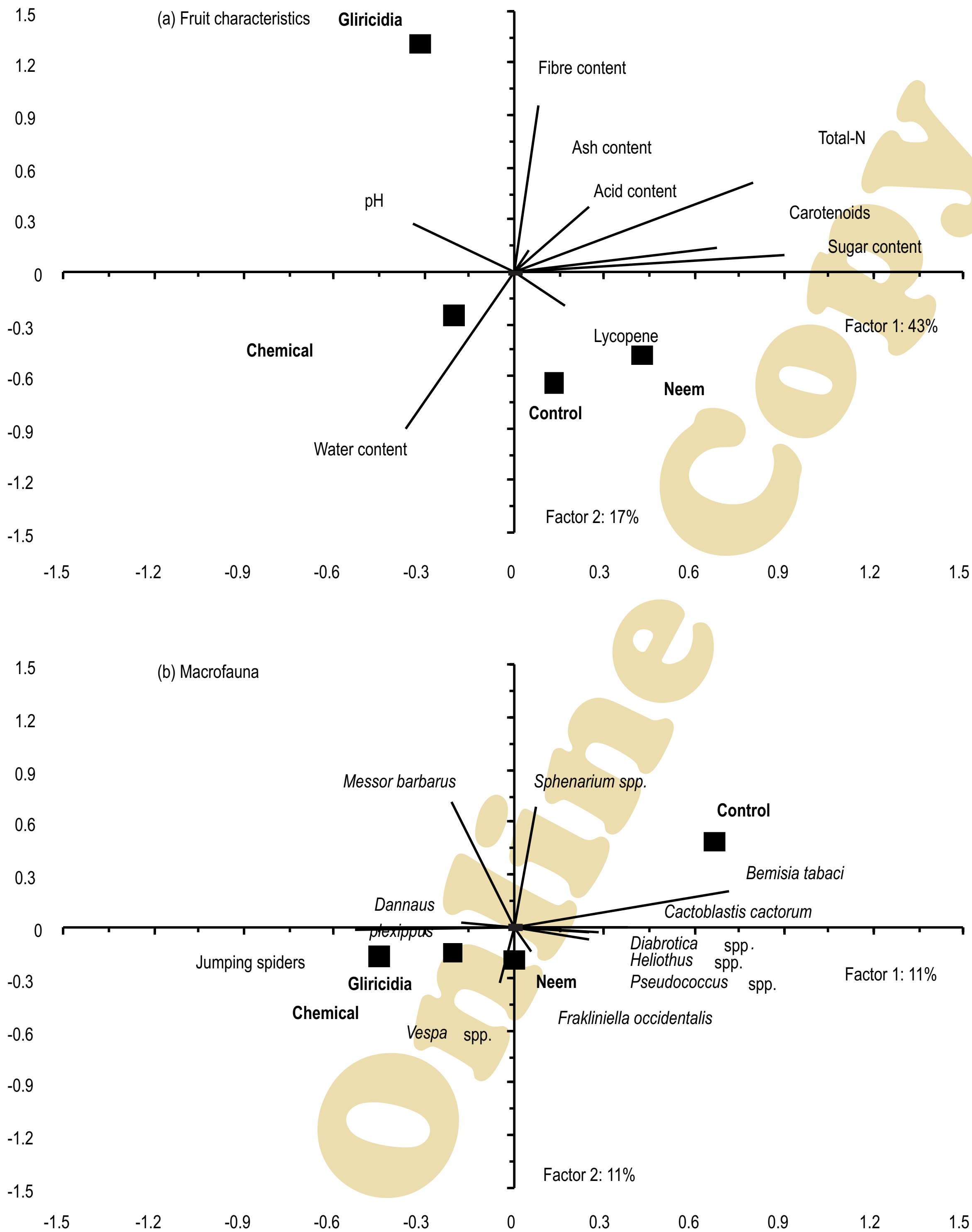

Fig. 2 : Principal component analysis of (a) tomato fruit characteristics; (b) macrofauna found on plants treated with lambda-cyhalothrin; leaf extracts of neem, mata-raton or left untreated (control treatment)

Applying leaf extracts of *G. sepium* to tomato plants increased their development and especially the dry and fresh weight. It is difficult to speculate what might have caused this, but different explanations are possible. First, the leaf extracts of *G. sepium* reduced the number of insects and worked as repellent so the damage to plants was smaller and they could develop better. However, this treatment was not a better repellent than leaf extracts of neem or chemical insecticide. Second, extracts of other plants, like eucalyptus (*Eucalyptus chamadulnosis* (Dehnh)), garlic (*Allium sativum*), marigold (*Tagetes erecta*) and neem have nematicidal effect on juveniles of nematode, *Meloidogyne incognita* (Lopes *et al.*, 2011). It might be that leaf extracts of *G. sepium* have also nematicidal effects or inhibit growth of other organisms (e.g. fungi) thereby aiding plant growth, but it is most unlikely that an extract sprayed on the foliage will affect soil organisms. Third, the leaf extracts of *G. sepium* contained nutrients that stimulated plant growth. For instance, the total N applied with neem extracts was higher (146 mg N l^{-1}) than applied with *G. sepium* extract (70 mg N l^{-1}), but C-to-N ratio was lower in the latter (13.2) than in the former (37.1). The extract of *G. sepium* might provide N to the growing tomato plants when the organic N is mineralized, while organic N mineralized when neem extract is applied is immobilized again. The total N in tomato plants was similar in the neem and Gliricidia treatments, so it appears that the amount of N applied was similar in neem and Gliricidia treatments. The plants in different treatments were fertilized in the same way so it did not affect plant growth. However, the extract of *G. sepium* contain microelements, such as sulphur, that might stimulate plant growth (Kandil and Gad, 2010). It is most unlikely, however, that the concentrations of microelements in the neem and mata-raton extracts are very different. Fourth, the leaf extracts of *G. sepium* contained a component that stimulated plant growth. It has been shown that grapes (*Vitis vinífera*) have the potential to synthesize abscisic acid a regulator of plant growth, *in situ* (Wheeler *et al.*, 2009). It could be that the leaf extracts of *G. sepium* contain ABA and when the extract is applied to the plant stimulates tomato growth. A fifth possible explanation is that the tomato plant itself produces ABA or another growth regulator as a consequence of stress induced by application of leaf extract of *G. sepium*. ABA-regulated gene expression, involved in desiccation tolerance, is expressed mainly in seeds and in vegetative tissues under stress, but its production has recently been demonstrated in tomato fruits (Bastías *et al.*, 2011).

Tomato fruit treated with leaf extracts of *G. sepium* were characterized by high fibre content and high concentration of lycopene and carotenoids, but low water content. Fibre are mostly found in the outer layers of tomato. Under water stress, the plants increase fibre content in the outer layers thereby reducing water loss. Spraying leaf extract of *Gliricidia* on tomato foliage might have induced salt stress or induced a yet unknown stress. This stress might induce production of certain components, e.g. sugars, to increase osmotic pressure in the cells, protecting the plants against water loss. High concentration of lycopene, sugars and carotenoids also indicated that tomato plants treated with *G. sepium* were under stress. Wu and Kubota (2008) reported that when tomatoes were cultivated in solutions with high electrolytic conductivity, the concentrations of lycopene, chlorophyll and sugars increased. This contributed to reduce water flow due to osmotic or salt stress. Increased concentration of lycopene, however, has an additional benefit. Consumption of tomato products has been associated with a decreased risk of developing prostate cancer, and lycopene, the red carotenoid in the tomato, is a potent antioxidant that might contribute to this chemoprevention activity (Van Breemen *et al.*, 2011).

Treatment had a similar effect on the characteristics of tomato leaves as on the characteristics of tomato fruits. Leaf extracts of *G. sepium* not only affected tomato fruits but also the characteristics of tomato leaves. Tomatoes treated with leaf extracts of *G. sepium* were characterized by high fibre, and low water content.

Treating plants with extracts of neem leaves had little effect on the development of tomato plants, and fruit and leaf characteristics under the given cultivation conditions as

Table 4 : The effect of leaf extracts of neem and mata-raton on fruit characteristics of tomato plants cultivated in greenhouse

Characteristics	**Control**[a]	**Gliricidia**[b]	**Neem**[c]	**Chemical**[d]	**MSD**[e]	**F value**	**P value**
Ash content (g kg^{-1})	9 a[f]	10[a]	7[b]	11[a]	2	14.46	<0.0001
Fibre content (g kg^{-1})	91[b]	154[a]	96[b]	88[b]	11	114.76	<0.0001
Total N content (g kg^{-1})	10.5[a]	13.0[a]	12.0[a]	10.6[a]	2.3	3.63	0.0147
Sugar content (g kg^{-1})	24[ab]	21[b]	27[a]	26[ab]	5	3.48	0.0177
pH	3.86[b]	4.12[a]	3.88[b]	4.03[ab]	0.21	4.78	0.0034
Acids (g kg^{-1})	1.9[c]	3.1[a]	2.2[b]	1.9[c]	0.1	4.77	0.0034
Lycopene (mg kg^{-1})	146.4[a]	113.8[b]	162.5[a]	164.3[a]	22.1	16.45	<0.0001
Carotenoids (mg kg^{-1})	167.9[b]	145[c]	201.8[a]	203.6[a]	17.6	34.70	<0.0001
Water content (g kg^{-1})	860[a]	800[b]	860[a]	860[a]	20	44.21	<0.0001

[a]Control : Plants that were exposed to insects but received no treatment, [b]Gliricidia: Plants treated with extracts of leaves of *Gliricidia*, [c]Neem: Plants treated with extracts of neem leaves, [d]Chemical: Plants treated with lambda-cyhalothrin, [e]MSD: minimum significant difference ($P < 0.05$), f values with same letter within the row are not significantly different between the treatments

compared to tomato plants treated with lambda-cyhalothrin or not treated at all. Treating plants with extracts of *G. sepium* leaves, however, stimulated plant growth and altered some fruit and leaf characteristics compared to tomato plants treated with lambda-cyhalothrin or not treated at all. Plant development might have increased as a result of growth hormones applied with the extract or extract induced growth hormones in tomato plant. Changes in the fruit characteristics indicated that extracts of *G. sepium* generated stress in tomato plants, which might have been a result of increased salt content or another yet unidentified factor. It was found that extracts of *G. sepium* improved the development of tomato plants, but further research is required to investigate the mechanisms involved. Although extract of neem is known to be an effective repellent for insects, it had no effect on insect populations in this study.

Acknowledgments

We thank E.R. Garrido-Ramirez and N. Espinoza-Paz for technical assistance. The research was funded by the '*Instituto Tecnológico de Tuxtla Gutiérrez*' (Chiapas, Mexico). V.M. R.-V. received grant-aided support from '*Consejo Nacional de Ciencia y Tecnología*' (CONACyT).

References

AOAC: Official methods of analysis, 14th ed. Association of Official Analytical Chemists (AOAC), Washington, DC., USA (1980).

Bastías, A., M. López-Climent, M. Valcárcel, S. Rosello, A. Gómez-Cadenas and J.A. Casaretto: Modulation of organic acids and sugar content in tomato fruits byan abscisic acid-regulated transcription factor. *Physiol. Plant.,* **141**, 215-226 (2011).

Boursier, C.M., D. Bosco, A. Coulibaly and M. Negre: Are traditional neem extract preparations as efficient as a commercial formulation of Azadirachtin A? *Crop Prot*., **30**, 318-322 (2011).

Diaz-Pendon, J.A., M.C. Canizares, E. Moriones, E.R. Bejarano, H. Czosnek and J. Navas-Castillo: Tomato yellow leaf curl viruses: menage a trois between the virus complex, the plant and the whitefly vector. *Mol. Plant Pathol.,* **11**, 441-450 (2010).

El Shafie, H.A.F. and T. Basedow: The efficacy of different neem preparations for the control of insects damaging potatoes and eggplants in the Sudan. *Crop Prot.*, **22**, 1015-1021 (2003).

INIFAP-CECECH: Manual de diagnóstico-prescripción para el cultivo de maíz en el estado de Chiapas. SARH, pp. 17–291 (1999).

Kandil, H. and N. Gad: Response of tomato plants to sulphur application in addition to organic fertilizer. *Int. J. Acad. Res.* **2**, 1-8 (2010).

Lopes, E.A., S. Ferraz, P.A. Ferreira, L.G. de Freitas and R. Dallemole-Giaretta: Soil amendment with chopped or ground dry leaves of six species of plants for the control of *Meloidogyne javanica* in tomato under greenhouse conditions. *Cienc. Rural,* **41**, 935-938 (2011).

Montes-Molina, J.A., M.L. Luna-Guido, N. Espinoza-Paz, B. Govaerts, F.A. Gutierrez-Miceli and L. Dendooven: Are extracts of neem (*Azadirachta indica* A. Juss.) and mata-raton (*Gliricidia sepium* (Jacquin) an alternative to control pests on maize (*Zea mays* L.). *Crop Prot.,* **27**, 763-774 (2008).

Ortega A.C.: Insect Pests of Maize: A Guide for Field Identification. CIMMYT ISBN. 968-6127-07-0. México, D.F (1987).

Pedersen, A.B. and L.D. Godfrey,: Field and vegetable crops as hosts of larval western spotted cucumber beetle (Coleoptera: Chrysomelidae). *Environ. Entomol.* **40,** 633-638 (2011).

Salako, E.A., S.T. Anjorin, C.D. Garba and E.B. Omolohunnu: A review of neem biopesticide utilization and challenges in Central Northern Nigeria. *Afr. J. Biotechnol.,* **7**, 4758-4764 (2008).

SAS Institute: Statistic guide for personal computers. Version 6.04, Edition. SAS Institute, Inc. Cary (1989).

Schaaf, O., A.P. Jarvis, S.A. van der Esch, G. Giagnavoco and N.J. Oldham: Rapid and sensitive analysis of azadirachtin and related triterpenoids from neem (*Azadirachta indica*) by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr.*, **886**, 89-97 (2000).

Sharma, N., J. Qadry, B. Subramanium, T. Verghese, S. Rahman, S. Sharma and S. Jalees: Larvicida activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephansi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Pharma. Biol.,* **36**, 3-7 (1998).

Valdez-Perez, M.A., F. Fernández-Luqueño, O. Franco-Hernandez, L.B. Flores-Cotera and L. Dendooven: Cultivation of beans (*Phaseolus vulgaris* L.) in limed or unlimed wastewater sludge, vermicompost or inorganic amended soil. *Sci. Hortic.,* **128**, 380-387 (2011).

Van Breemen, R.B., R. Sharifi, M. Viana, N. Pajkovic, D. Long, P.E. Yuan-Bowen, and M. Stacewicz-Sapuntzakis: Antioxidant effects of lycopene in African American men with prostate cancer or benign prostate hyperplasia: A randomized, controlled trial. *Cancer Prev. Res.,* **4**, 711-716 (2011).

Villar, S.B. and S.E. López: Negro Grijalva. Nueva variedad de frijol para Chiapas y regiones similares. Folleto Técnico Agrícola No. 2. INIFAP. Ocozocoautla de Espinoza Chiapas, México (2003).

Wang, Z.F., T.J. Ying, B.L. Bao and X.D. Huang: Characteristics of fruit ripening in tomato mutant epi. *J. Zhejiang Univ. SCI.,* B **6**, 502-507 (2005).

Webster, R., Statistics to support soil research and their presentation. *Eur. J. Soil Sci.,* **52**, 331-340 (2001).

Wheeler, S., B. Loveys, C. Ford and C. Davies: The relationship between the expression of abscisic acid biosynthesis genes, accumulation of abscisic acid and the promotion of *Vitis vinifera* L. berry ripening by abscisic acid. *Aust. J. Grape Wine Res.,* **15**, 195-204 (2009).

Wu, M., and C. Kubota: Effects of high electrical conductivity of nutrient solution and its application timing on lycopene, chlorophyll and sugar concentrations of hydroponic tomatoes during ripening. *Sci. Hortic.,* **116**, 122-129 (2008).