OCL 2021, 28, 32

https://doi.org/10.1051/ocl/2021018

Available online at:
www.ocl-journal.org

Research Article Open Access

Relative toxicity of two natural compounds compared to abamectin against some soybean pests under period rates

Hala Hussien Alakhdar[1,*] and Zeinab Elsayed Ghareeb[2]

[1] Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt
[2] Central Laboratory for Design and Statistical Analysis Research, Agriculture Research Center, Giza, Egypt

Received 9 September 2020 – Accepted 22 March 2021

Abstract – A sustainable pest management in agro-ecosystems requires parallel assessments of pesticide and natural compounds to control target pests. In the present study, a semi-field experiment was conducted to evaluate the relative toxicity of abamectin (Abamax), humic acid, and Chitosan Nano-Particles (C.N.Ps) against four soybean pests: *Tetranychus urticae*, *Eutetranychus orientalis*, *Bemisia tabaci*, and *Phenacoccus solenopsis*. The experimental treatments were arranged in a split-plot design with three replicates, where treatments were assigned to main plots and pest populations under different periods in the subplots. The obtained results and graphs demonstrated that there were considerable differences between the total numbers of pests after different periods of treatment. Generally, *E. orientalis* recorded the minimum pest number at all. Different tested compounds demonstrated a considerable correlation between the densities of the pests. *T. urticae* exhibited a significant correlation with the other three pests (*E. orientalis*, *B. tabaci*, and *P. solenopsis*). *E. orientalis* did not correlate with *B. tabaci* and *P. solenopsis*. The humic acid recorded the best effect on *T. urticae* after 3 days with a reduction of 85.45% and *E. orientalis* after 7 days 65.55%. However, Chitosan Nano-Particles (C.N.Ps) was the best for *E. orientalis* after 14 days (mortality 74.36%). In contrast, abamectin (Abamax) had a general mean of reduction of 91.17% against *T. urticae*, whenever, these compounds are promising for controlling *T. urticae*, *E. orientalis*, *B. tabaci*, and *P. solenopsis*. These results may be a supporting method to overcome some soybean pests. The findings are discussed within the context of integrated management of soybean pests under semi-field conditions.

Keywords: soybean / *Tetranychus urticae* / *Eutetranychus orientalis* / *Bemisia tabaci* / *Phenacoccus solenopsis* / biplot / abamectin / humic acid / Chitosan Nano-Particles

Résumé – Ravageurs du soja : efficacité à différentes dates de 2 composés naturels comparés à l'abamectine. La gestion durable des ravageurs dans les agro-écosystèmes nécessite des évaluations comparées des pesticides et des composés naturels pour contrôler les ravageurs cibles. Dans la présente étude, une expérience en semi-champ a été menée pour évaluer l'efficacité relative de l'abamectine (Abamax), de l'acide humique et des nanoparticules de chitosan (C.N.Ps) contre quatre ravageurs du soja : *Tetranychus urticae*, *Eutetranychus orientalis*, *Bemisia tabaci* et *Phenacoccus solenopsis*. Le dispositif expérimental mis en place est un *split-plot*, avec trois répétitions ; les traitements ont été assignés aux blocs principaux et les populations de ravageurs à différentes périodes aux sous-blocs. Les résultats obtenus et les graphiques ont démontré qu'il y avait des différences considérables entre les nombres totaux de ravageurs après différentes périodes de traitement. En général, *E. orientalis* était le ravageur le moins présent. Les différents composés testés ont démontré une corrélation considérable entre les densités de ravageurs. *T. urticae* a montré une corrélation significative avec les trois autres ravageurs (*E. orientalis*, *B. tabaci*, et *P. solenopsis*). *E. orientalis* n'a pas présenté de corrélation avec *B. tabaci* et *P. solenopsis*. Les meilleures efficacités de l'acide humique sur *T. urticae* sont enregistrées après 3 jours avec une réduction de 85,45 % et sur *E. orientalis* après 7 jours (65,55 %). Cependant, les meilleurs résultats sur *E. orientalis* avec les nanoparticules de chitosan (C.N.Ps) ont été obtenus après 14 jours (mortalité de 74,36 %). En revanche, l'abamectine (Abamax) a réduit en moyenne de 91,17 % *T. urticae*. Ces composés sont donc prometteurs pour lutter contre *T. urticae*, *E. orientalis*, *B. tabaci* et *P. solenopsis*. Ces résultats

*Correspondence: hala_alakhdar@yahoo.com

montrent que les composés naturels testés pourraient constituer des méthodes de lutte alternatives sur certains ravageurs du soja. Les résultats sont discutés dans le contexte de la gestion intégrée des ravageurs du soja dans des conditions de semi-liberté.

Mots clés : soja / *Tetranychus urticae* / *Eutetranychus orientalis* / *Bemisia tabaci* / *Phenacoccus solenopsis* / biplot / abamectine / acide humique / nanoparticules de Chitosan

1 Introduction

Soybean (*Glycine max*) is considered an important oilseed crop that has grown in the world (60% of world oilseed production) (US Soybean Export Council, 2019). About 15 000 ha was sown soybean in Egypt, total production was 48 000 tons (FAO, 2018). Numerous kinds of pests attack soybean from seedling to mature stages such as spider mites, aphids, cotton leaf worms, and many other pests. Pests' infestation can lead to yield losses from 20 to 50%. Therefore, the farmers use pesticides to protect their crops (Fikru and Leon, 2003; Massoud *et al.*, 2014).

Polyphagous pests are wide-ranged on many economic and important crops including soybean (Alakhdar *et al.*, 2015). The two-spotted spider mite *Tetranychus urticae* Koch, and the oriental red mite *Eutetranychus orientalis* Klein are piercing-sucking pests' infest soybean plant. This behavior of feeding leads to the appearance of characteristic yellow-chlorate spots on the leaves. Pale yellow streaks develop along the midrib and veins initially, which later progress to a grayish or silvery appearance of the leaves. In heavier infestations, mites feed and oviposit over the whole surface of the leaf, causing leaf fall and die-back of branches, which may result in defoliated plants (Moghadam *et al.*, 2016). Two insect species also attack soybean and cause a serious economic problem. The whitefly, *Bemisia tabaci*, can decrease plant health and yield by removing large amounts of plant photosynthetic from the leaves, heavy infestations in young plants can reduce plant growth; later infestations can drop off the number of pods set, and seed size consistency, thus reducing yield and quality. The invasive mealybugs *Phenacoccus solenopsis* is a highly invasive and polyphagous insect responsible for serious damage to crops and plants. It attacks more than 200 plant species including field crops, vegetables, ornamentals, and weeds, bushes, and trees. (Arif *et al.*, 2009; Fand and Suroshe, 2015).

Pests are controlled using traditional methods such as chemical pesticides. The need to study the correlation between the efficacy of pesticides and other natural alternatives on several target and non-target pests, to reduce their populations, is inescapable. Abamectin belongs to the family known as macrocyclic lactones, which is one of the pesticides applied to control pests. It is often used as an insecticide, acaricide, and nematicide and is found under many commercial names such as Abamax, Vertimec, etc. (Lasota and Dybas, 1990; Alhewairini, 2018). Most of the chemical pesticides are not only expensive but also kill natural enemies. Moreover, the use of these pesticides leads to serious threats such as the resurgence of insects (Kumari *et al.*, 2015), the development of resistance in insects, environmental pollution, and harmful consequences on human health. Many studies have determined the importance of using natural alternatives in integrated pest management programs, one of them is humic acid, which is, a commercial product containing many elements that increase the availability of nutrients and consequently increase plant growth, mineral nutrition, seed germination, seedling growth, root initiation, root growth, shoot development, and the uptake of macro and microelements. Moreover, humic acid considered to induce resistance of plants against some pests (Çelik *et al.*, 2011, Xu *et al.*, 2015; Ekin, 2019; Alakhdar *et al.*, 2020). Chitosan Nano-Particles (C.N.Ps) is a new natural compound that was derived from chitin (Gan *et al.*, 2005). It shows strong insecticidal and acaricidal activities and may serve as a good alternative for broad-spectrum and highly persistent pesticides. C.N.Ps has a potential for biological control of several pests with a slight effect on some associated natural enemies.

Therefore, the knowledge of the relationship between these pests under different treatments, obtainable through correlation coefficient, was detected to measure only the degree (intensity) and nature (direction) of association (Golkar *et al.*, 2011). The problem gets complicated in selection studies especially when there is a negative interaction between the primary trait of the experiments and the other traits (De Leon *et al.*, 2016) or treatments. Recently, GGE (Genotype plus Genotype by Environment) biplot method was developed by Yan (2014) to use different types of biplot graphs created to discuss the effects of applied treatment on one or all target traits at the same time, allowing the user to assess entire two-way data (Gabriel, 1971). Assessments are usually performed over PC1 and PC2 (the first two principal components) axes calculated from the data of rows and columns from a two-dimensional array produced by the combination of treatment and traits datasets (Akcura and Kokten, 2017).

In Egypt, no reference was found considering the technique of treatment by pest (TP)-biplot graph. Therefore, the objective of the current work was to:

1. Evaluate the efficacy of using natural compounds such as humic acid, and Chitosan Nano-particles, compared to an acaricide Abamectin: Abamax, after different treatment periods against target pests, *Tetranychus urticae* and *Eutetranychus orientalis* and non-target pests, *Bemisia tabaci*, and *Phenacoccus solenopsis*.
2. Study the interrelationships among pests infestations' using the (TP)-biplot technique.

2 Materials and methods

A semi-field experiment was conducted at an experimental farm known as Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt during the 2nd week of June in the two successive summer seasons of 2019 and 2020 on soybean. Crawford variety was kindly provided by Field Crops Research Institute (FCRI) to study the effect of three compounds – a commercial insecticide (Abamectin: Abamax), an organic compound (humic acid), and a nanomaterials (Chitosan Nano-Particles) – after periods (3, 7 and 14 days of spraying) to reduce the populations' density of *Tetranychus urticae*, *Eutetranychus orientalis*, *Bemisia tabaci*, and *Phenacoccus solenopsis*.

Table 1. Different tested treatments of the experiments (compounds, pests, and periods) and their characteristics.

Factors	Name	Characteristics
Pests mites/insects	Two-spotted spider mite	*Tetranychus urticae* Koch, (Acari: Tetranychidae)
	Oriental red mite	*Eutetranychus orientalis* Klein, (Acari: Tetranychidae)
	Whitefly	*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae).
	Mealybugs	*Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)
Spray compounds: Materials	Humic acid	Humic acid 12%, 60 cm^3/fed. Obtained from Central Laboratory of Organic Agriculture/Agricultural Research Center.
	Nano-Chitosan Particles (C.N.Ps.)	C.N.Ps were prepared according to Gan *et al.* (2005), the used concentration was the LC_{90}133.3 ppm. recommended for *T. urticae* (Alakhdar, 2020).
	Abamectin Abamax	Trade name: Abamax® 1.8% E.C., 40 cm^3/100 L.
Time	Before	Before spraying
	3 days	Assessing the effect after 3 days
	7 days	Assessing the effect after 7 days
	14 days	Assessing the effect after 14 days

2.1 Experiments procedure

Experimental treatments were arranged in a split-plot design with three replications; treatment compounds were assigned to main plots and the pest populations in the subplots. The selected area of about 1.4 kirats (Kirat = 175 m^2) was split into 6 plots and controlled each plot consisted of 6 ridges, 70 cm apart, and 4 m long. Three rows of soybean plants, between treatments, were not sprayed as barrier zones to avoid drift spray between treatments. Seeds were sown in the first week of May during the two seasons. Abamectin and humic acid were sprayed with the recommended dose rate, while Chitosan Nano-Particles (C.N.Ps) was applied with as suggested by one of the author's Lc_{90} (133.3) ppm of *T. urticae* (Alakhdar, 2020), one plot was sprayed with water as a control (Tab. 1). Moreover, all other cultural practices were applied as recommended.

2.2 The procedure of Bioassay and data recording

After the infestation of pests was confirmed, a pre-spray sample was taken and the treatment was carried out with all tested compounds. Ten leaves were taken from each replicate and the number of each pest, all movable stages of *Tetranychus urticae*, *Eutetranychus orientalis*, *Bemisia tabaci*, and *Phenacoccus solenopsis*, was counted before treatment and after 3, 7, and 14 days post-treatment by the aid of a stereomicroscope in acarology lab. at PPRI (Alakhdar, 2020). The reduction percentages of the pest's population in all treatments compared to the control was calculated according to the Henderson and Tilton formula (Henderson and Tilton, 1955):

Population reduction =

$$\text{Corrected\%} = \left(1 - \frac{\text{n in Co before treatment} * \text{n in Tafter treatment}}{\text{n in Co after treatment} * \text{n in Tbefore treatment}}\right) * 100,$$

where n = insect population, T = treated and Co = control.

The performance of the number of each pest under the tested treatments (Tab. 1) was obtained.

2.3 Statistical analysis

Data on individual pests was carried out on the mean values over three replications. At first, the analysis of variance was applied, and then a combined analysis of variance was computed over two seasons according to Sendecor and Cochran (1981). Before running the combined analysis, Levene (1960) test was used to satisfy the assumption of homogeneity of variances. The mean comparison was done using the least significant differences test at a 5% level of probability. The number of pests data was transformed according to $(x+1)^{1/2}$ and was applied to detect statistical differences among pests numbers. The transformed data analysis can modify the coefficient of variation (C.V. %). Correlations among different pests' data were subjected, according to Sendecor and Cochran (1989), to reveal the relationship among soybean pests. When F was significant ($P < 0.05$) for the levels of symptoms analysis was performed. The genotype by trait (GGT) biplot, which is an application of the GGE biplot used to study the genotype by trait data (Yan and Rajcan, 2002). The biplot method was employed to display the treatment by trait (TT) two-way data in the biplot graph and denoted as treatment-pest (TP)-biplot graph according to Akcura and Kokten (2017), using GenStat software (18.0 version) by ICARDA.

3 Results and discussion

3.1 Efficacy of compounds against different pests

Reduction percentages were calculated for each treatment, showing the effect of sprayed compounds (humic acid, C.N.Ps, and abamectin) against movable stages of different pests (*T. urticae*, *E. orientalis*, *B. tabaci*, and *P. solenopsis*) after 3, 7, and 14 days of treatment under natural conditions (Fig. 1). All compounds indicated a decrease in the number of mites/insects

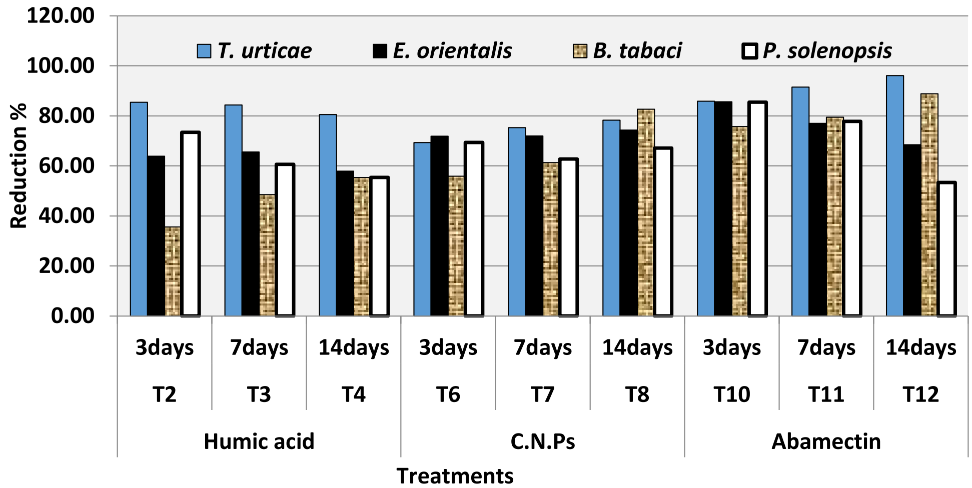


Fig. 1. Reduction index (RI) of spray compounds against movable stages of different pests after 3, 7, and 14 days of treatment under natural conditions.

compared with control treatment after different periods. Hence, the formula of Henderson and Tilton (1955) was used to calculate the percentage of pest population reductions using the mean population pre- and post-spraying in treated and untreated controls. Results indicated that humic acid had a mortality percentage for *T. urticae* (85.45, 84.4, and 80.5%) and *E. orientalis* (63.91, 65.55, and 57.89%) after 3, 7, and 14 days of treatment, respectively. The highest effect of humic acid was obtained for *T. urticae* after a different period and followed by *P. solenopsis* (73.43%) after 3 days. Meanwhile, it had the lowest effectual impact on *B. tabaci* (35.6 and 48.26%) after 3 and 7 days of treatment, respectively. C.N.Ps. revealed the highest mortality effect only on *T. urticae*, recording 75.3 and 74.36% after 7 and 14 days, respectively. However, C.N.Ps revealed the highest mortality effect on *E. orientalis* under different treatments, recording 75.3, 74.63, and 71.86% after 14, 7, and 3 days, respectively. Moreover, abamectin had an efficacy on *T. urticae* recording values above 90% (96.1, 91.52, and 85.9% after 3, 7, and 14 days, respectively) and for *E. orientalis* and *P. solenopsis* (85.6 and 85.5) after 3 days, respectively followed by *B. tabaci* (88.70) after 14 days. Therefore, all three spraying compounds (humic acid, C.N.Ps., and abamectin) had the highest mortality effects against *T. urticae* after different periods except for C.N.Ps treatment after 3 days. However, *B. tabaci* had the lowest motility effect for humic acid after 3 and 7 days (Tab. 2).

The three spraying compounds (humic acid, C.N.Ps., and abamectin) indicated a reduction in the mean number of pests under study in variance responses. There is a good match between our results and Prabhat and Poehling (2007) who reported heavy reduction percentages on the three nymphal stages of *B. tabaci* treated with abamectin within 24 h post-application, while the first instars being most susceptible. Kenneth *et al.* (2002) indicated that the mortality from abamectin residues was not significantly greater than the control at one day after application, but it was significantly greater than the control after 3, 7, and 14 days of treatment. Few studies recorded the effect of acaricides against *E. orientalis*, Alhewairini found that the populations of *E. orientalis* reduced to 76.68 and 78.52 after one-week exposure to the recommended dose (RD) of abamectin under field and laboratory conditions (Márquez *et al.*, 2006; Alhewairini, 2018). The increase of the mean reduction in the population of *T. urticae* and *E. orientalis* may due to the specialty of abamectin: Abamax, as acaricide has efficacy on all stages of mites. Whenever its effect on *P. solenopsis* decreased over time as most of the insecticides used are mixed abamectin with another formula (Rezk *et al.*, 2019).

Particular attention is paid to humic acid, as an organic compound that usually provides plants with a balanced source of nutrients that can influence the composition and physiology of plants. Apart from that, it might have provided some growth-promoting substances, vitamins and these probably have increased the plant resistance to pests or made the plants less palatable to the pest. It emerged superior in minimizing the whitefly 75% compared with other used compounds (Chatterjee *et al.*, 2013). On other the hand, Panda *et al.* (2005) reviewed the lowest population of sucking pests, jassids, and thrips in chilli. The mechanisms for decreasing pest attacks may be due to the differential availability of mineral nutrients in plants, which might have enhanced the induced resistance development and subsequently helped in escaping sucking-piercing pest infestation. Furthermore, organic treatments reduced the incidence of sucking pest as whitefly and leafhopper that organic amendments comparatively increased the total phenols in the plants and also the activity of the enzymes like polyphenol oxidase and peroxidase, which might be responsible for the reduced pest incidence (Ravi *et al.*, 2006). Consequently, more researches are needed on the mode of action and compatibility of tested compounds with bio- and organic-origin agents (Alakhdar *et al.*, 2020).

A similar approach is used for two tetranychid mites, *Tetranychus urticae* (Koch) and *Tetranychus cinnabarinus*, and their eggs and immature stages on dry beans (*Phaseolus vulgaris* L.). It was found that Chitosan Nano-Particles (C.N.Ps) is potent against *T. urticae* (Alakhdar, 2020). Other studies carried on C.N.Ps to evaluate its insecticidal effect on other pests, and Zhang and Tan (2003) reported that Chitosan exhibited different insecticidal activity to various aphids ranged between 93 and 99% for *Hyalopterus pruni* (Goffroy)

Table 2. Reduction percentages % according to Henderson and Tilton's formula as the effect of different treatment under natural conditions.

Treatments/Pests		Period/days	*T. urticae*		*E. orientalis*		*B. tabaci*		*P. Solenopsis*	
			c	%	c	%	c	%	c	%
Humic acid	T1	BT	195		38		152		177	
	T2	3	32	85.45	10	63.91	72	35.61	110	73.43
	T3	7	38	84.41	6	65.55	98	48.26	199	60.65
	T4	14	58	80.5	8	57.89	167	55.36	261	55.36
		Mean		83.45		62.45		46.41		63.15
C.N.Ps	T5	BT	208		39		122		127	
	T6	3	72	69.36	8	71.87	57	55.84	91	69.36
	T7	7	64	75.3	5	72.03	59	61.36	135	62.79
	T8	14	69	74.36	5	74.36	52	82.7	138	67.1
		Mean		73.0		72.75		61.79		66.63
Abamectin	T9	BT	151		38		109		139	
	T10	3	24	85.9	4	85.6	28	75.7	47	85.5
	T11	7	16	91.52	4	77	28	79.47	88	77.84
	T12	14	9	96.1	6	68.42	30	88.8	214	53.39
		Mean		91.17		77		81.32		72.24

BT = before treatment; c = count of motile stages.
T1: before spraying humic acid; T2: 3 days after spraying humic acid; T3: 7 days after spraying humic acid; T4: 14 days after spraying humic acid; T5: before spraying C.N.Ps; T6: 3 days after spraying C.N.Ps; T7: 7 after spraying C.N.Ps; T8: 14 days after spraying C.N.Ps; T9: before spraying abamectin; T10: 3 days after spraying abamectin; T11: 7 days after spraying abamectin; T12: 14 days after spraying abamectin.

Table 3. Mean squares of combined analysis of variance for a different number of pests under spray compounds and Periods rates.

S.O.V.	d.f.	*T. urticae*	*E. orientalis*	*B. tabaci*	*P. Solenopsis*
Year (Y)	1	300.125*	34.72**	115.01	144.50
Error	4	113.49	4.31	347.97	1008.11
Spray compound (Sc)	2	2444.68**	2.06*	1217.93**	24 110.18**
Sc * Y	2	117.54	0.22	42.18	344.54
Error	8	47.86	3.47	52.66	779.69
Periods (P)	3	1192.83**	5.72**	281.50**	2212.65**
P * Sc	6	980.9**	0.83	389.63**	2154.05**
P * Y	3	179.90*	0.76	72.76	375.46
Error	42	60.69	0.54	46.71	258.18

* and **: Significant at 5 and 1% probability levels, respectively.

on flowers, while (Rabea *et al.*, 2005) tested the insecticidal activities of Chitosan Nano-Particles against larvae of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The same trends were also observed against *Aphis gossypii*; the mean number of eggs/female of *A. gossypi* was significantly decreased to 20.9 and 28.9 eggs/female compared with 97.3 and 90.3 of the non-treated controls, under laboratory and semi-field conditions, respectively (Sahab *et al.*, 2015).

3.2 Combined analysis and mean performance

Variances homogeneity for the studied number of pests in each mite/insect was confirmed according to the Levene (1960) test, which allowed the combined analysis. Accordingly, the mean variability for different treatments on each pest over the two seasons 2019 and 2020 were presented in Tables 3 and 4. Results showed that years affected significantly both *T. urticae* and *E. orientalis*. As well, significant differences among the different compound treatments for all pests except *E. orientalis* were obtained. Each of *T. urticae*, *B. tabaci,* and *P. solenopsis* revealed highly significant differences. Our results are in harmony with Sabbour (2016) and Alakhdar (2020) who reported that spray Chitosan Nano-Particles compounds had a high effect on pests' number on soybean. Concerning periods, highly significant differences were detected for all pests, which demonstrated the existence of a high effect of different periods. The results in this experiment

Table 4. Simple correlation coefficients among studied pests in the soybean field ($n = 144$).

Trait	*T. urticae*	*E. orientalis*	*B. tabaci*
E. orientalis	0.494** 0.000		
B. tabaci	0.383** 0.007	0.009 0.952	
P. solenopsis	0.224 0.126	0.064 0.668	0.436** 0.002

Cell Contents: Pearson correlation

* and **: Significant and high significant at probability levels 0.05 and 0.01, respectively.

are in agreement with the results of other researchers such as Ekin (2019) and in terms of the interaction between spray compound treatments and periods, there were highly significant differences for all the traits except *E. orientalis*.

3.3 Effect of tested compounds and periods rates

Figure 2 illustrated the effect of tested compounds on the studied pests on soybean over two seasons. Meanwhile, the analysis showed that this data wasn't subjected to normal distribution. Then data of the number of pests trait was transformed according to $(x+1)^{1/2}$ and reanalyzed for modifying analysis and coefficient of variation (C.V.).

The above results on soybean, the 1st compound (humic acid) recorded the best effect for *T. urticae* and *E. orientalis*, meanwhile, 2nd (Chitosan Nano-Particles) was the best for *B. tabaci* and *P. solenopsis*. Whenever *E. orientalis* recorded the minimum number at all. These results agreed with Ekin (2019).

3.4 Interaction effect of spray compounds and periods

Figure 3 represents the significant effects of the interaction between sprayed compounds and periods on the number of mites/insects. Data revealed that the humic compound had effects on *T. urticae* and *E. orientalis* mites, Chitosan nanoparticles (C.N.Ps) decreased *E. orientalis* and abamectin compound had effects on each from *T. urticae*, *E. orientalis*, and *B. tabaci* pests. It could be noticed that the combination in the application of humic acid, C.N.Ps, and abamectin may reduce pests' infestation in soybean plants when applied at this stage of plant growth. This time is accurate to manage pests understudy meanwhile keep their abundance under Economic Threshold (Alakhdar *et al.*, 2015; Czepak *et al.*, 2018; Abd El-Razzik, 2018; Mesbah *et al.*, 2019).

3.5 Correlation between pests in the soybean field

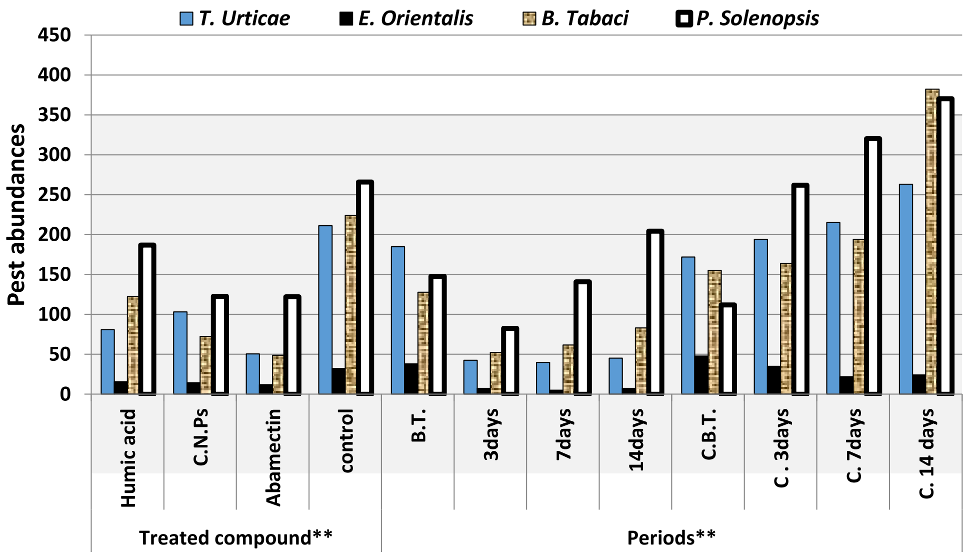
Generally, many pests were noted in the soybean field. Adequate knowledge of the relationship that exists between these pests is essential for the identification of infestation in soybeans. The correlation coefficients among all pairs of studied pests of soybean over the two seasons are given in Table 4. The results showed that there was a highly significant positive correlation between *T. urticae* and each of *B. tabaci* (0.474**) and *P. solenopsis* (0.323**). As well as, mealybugs, *P. solenopsis* had a highly significant positive correlation with the whitefly, *B. tabaci*. Meanwhile, *Eutetranychus* (0.302*) had only a significant positive association with *E. orientalis*. On the other hand, *E. orientalis* exhibited an insignificant correlation with *B. tabaci* and *P. solenopsis*. A significant correlation between these pests indicated that the simultaneous infestation of these pests is possible. These findings indicate that the efficiency of spraying compounds for each pest would be accompanied by a high effect of another pest.

3.6 Treatment pest biplot (combined data)

Generally, the biplot graphs, according to (Yan *et al.*, 2000; Yan, 2002; Yan and Rajcan, 2002; Yan and Tinker, 2005), can be used to compare genotypes in different environments (GE), genotypes based on multiple traits (GT) or treatment based on multiple traits (TT). The current study depended on the estimation of biplot polygon and vector graphs to study the effects of the used treatments (T) on the studied pests (P) in one graph which is termed as (TP)-biplot graph. This method that uses a combination of treatment and pest datasets is similar to the method of comparing treatments on multiple traits (Yan, 2002; Akcura and Kokten, 2017). The mean values of the effects of three compounds and four dates of accounting numbers of (mite-insects)/each pest (representing 12 factorial treatment combinations) were graphically summarized as shown in the polygon view (Fig. 4). The (TP)-biplot graph gives an overall picture of the interrelationships among factorial treatment and all pests simultaneously.

The treatment × pest (TP)-biplot model is generated according to Yan and Rajcan (2002). The polygon (which-won-where) view of the treatment × pest (TP)-biplot graph is a good tool to interpret the behavior pattern of treatment toward pest provided. Then, the biplot should explain a sufficient amount of the total variation. The principle components (PC) analysis based on (TP)-biplot method together explained that there is about 94.22% of the observed variation for the measured pests on soybean across studied treatments (spraying compounds by period's measurements). The first and second principle components (PC) explained 76.29% and 17.92%, respectively, and the cumulative variance of the first two PCA was found 94.22%. The first two PC's reflected more than 60% of the total variation. Therefore, it achieved the goodness of fit for the biplot model.

The polygon-view of the (TP)-biplot graph in Figure 4 indicated which spraying compound by period treatment combinations had the best values for which pests and correlated pests by mega-environment. Mega-environment identification is among the most important objectives of multi-environment statistical trials. The (TP)-biplot showed the variation of the twelve treatments in terms of four pests, treatments as bests ones for single or multiple pests, and grouped the twelve treatments based on pests that make them potential performances. On the right part of the graph (with the



B.T. before treatment; C.B.T. control before treatments; C.3 days control after 3 days; C. 7days control after 7 days; C14 days control after 14 days

Fig. 2. Mean performance of the number of mites/insect traits under spray compounds and period's treatments (Combined). B.T.: before treatment; C.B.T.: control before treatments; C.3 days control after 3 days; C.7 days control after 7 days; C.14 days control after 14 days.

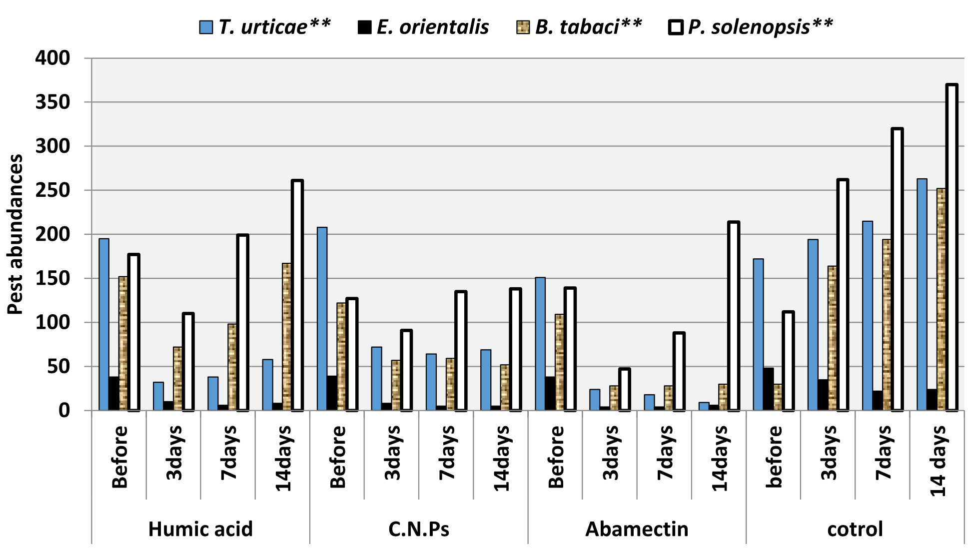


Fig. 3. Mean performance of the number of pests under the interaction between spray compounds and periods (combined).

relatively highest pest number), showing which treatments had the best values for the efficiency of pests. Four treatments T9, T11, T10, and T12 were the highest performing efficiency treatment for all pests (recording lowest insect number). The vertex treatment T11 (Abamectin: Abamax spraying compound after 7 days) on the right of the graph (positive part I) had the best, especially for *P. solenopsis* as the nearest pest for this treatment and *B. tabaci*. T10 (Abamectin: Abamax spraying compound after 3 days) and T12 (Abamectin: Abamax spraying compound after 14 days) which were middle between all pests (*T. urticae*, *E. orientalis*, *B. tabaci*, and *P. solenopsis*) recorded the best effective ones on pests. Meanwhile, T9 (before spraying Chitosan Nano-Particles compound), the best treatment has obtained for all pests, especially *T. urticae*. Then, the results of all treatments in the right part of the graph (T11, T12, T9, and T10) revealed that the abamectin effect with the highest insect number for all pests, especially *P. solenopsis* and *B. tabaci*. Therefore, this similarity established a strong correlation between *P. solenopsis* and *B. tabaci* pests in treatment results (as shown in Fig. 4).

The left part of the graph revealed the relatively lowest pest number. Regarding treatments, T2 (humic acid spraying compound after 3 days) and T3 (humic acid spraying compound after 7 days) were the vertex treatment on the left

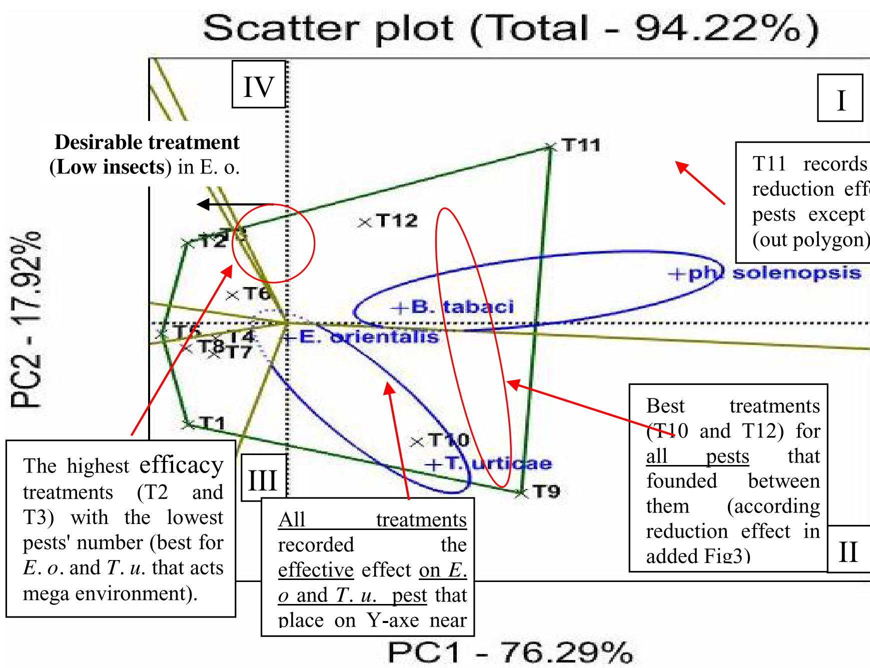


Fig. 4. Polygon-view of (TP)-biplot, showing which treatments had best values for which pests and mega-environment (correlated pests). T1: before spraying humic acid; T2: 3 days after spraying humic acid; T3: 7 days after spraying humic acid; T4: 14 days after spraying humic acid; T5: before spraying C.N.Ps; T6: 3 days after spraying C.N.Ps; T7: 7 days after spraying C.N.Ps; T8: 14 days after spraying C.N.Ps; T9: before spraying abamectin; T10: 3 days after spraying abamectin; T11: 7 days after spraying abamectin; T12: 14 days after spraying abamectin.

part of the graph for the *E. orientalis* mite. Therefore, T2 and T3 (humic acid spraying compound after 3 and 7 days) recorded the best treatments for *E. orientalis* with a similar effect was obtained. *T.urticae*, showing the strong correlation between *E. orientalis* and *T. urticae* results as shown in (TP)-biplot graph (Fig. 4) and Table 4. Therefore, all treatments on the negative part of the graph recorded the lowest number of pests, then, it was considered as the best treatment and had a good effect for all pests (Yan and Hunt, 2002; Yan and Rajcan, 2002).

The (TP)-biplot graph displayed the relationship among the four pests on soybean. Also, traits with longer vectors are more responsive to the treatment combinations and traits with shorter vectors are less responsive to the treatment combinations as well as those located at the biplot center are not responsive at all (Yan and Hunt, 2002; Yan and Rajcan, 2002; Yan and Frégeau-Reid, 2008). Then, the ideal test trait (pest) effectively discriminates treatments and represents their grouping which can be classified as pests with low treatment discrimination that should be selected as test trait number of insects/mites. In addition to the results of the traditional method of analyzing data, biplot provides more information on the effectiveness of the treatments with the view of identifying the ideal (best) one or pest. Most of the above findings can be verified from the original correlation coefficients.

4 Conclusion

The present study revealed the reduction of chemical pesticides through judicious application of bio and organic compounds, Chitosan Nano-Particles (C.N.Ps), and humic acid was tested. Abamectin (Abamax) is a recommended pesticide compared to these natural compounds against *T. urticae*, *E. orientalis*, *B. tabaci,* and *P. solenopsis* on soybean crops. The obtained results demonstrated that there were considerable differences between the total numbers of these pests after different periods. There was a highly significant correlation between these pests indicated that simultaneous infestation of these pests is possible. These findings indicate that the efficiency of spraying these compounds for each pest would be accompanied by a high effect on another pest. Based on the modified treatment-by-pest biplot analysis, it was concluded that the treatments (three spraying compounds by four-period interactions) were identified as effective treatments for pests and these treatments would be considered as key during the selection. Biplot method of treatment × pest (TP) together established a significant correlation between *P. solenopsis* and *B. tabaci* pests in treatment and a significant correlation between *E. orientalis* and *T. urticae* results as shown in the (TP)-biplot graph. Treatments on the negative part of the graph recorded the lowest number of pests, then, it was considered as the best treatment and had a good effect on all pests. As similar other results were shown. Then, the biplot graph gave a conclusion for all treatments of all pests.

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Cite this article as: Alakhdar HH, Ghareeb ZE. 2021. Relative toxicity of two natural compounds compared to abamectin against some soybean pests under period rates. *OCL* 28: 32.