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Research Article

Field Evaluation of New Formulation Types of Essential Oils against *Tuta absoluta* and their Side Effects on Tomato Plants

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Abstract

The Physico-chemical properties of four prepared tested formulations, namely Basi (basil oil, *Ocimum basilicum*), Cura (Turmeric oil, *Curcurma longa*), Rosa (rosemary oil, *Rosmarinus officinalis*) and Thymo (thyme oil, *Thymus vulgaris*), compared with Nimbecidine commercial insecticide were measured. The efficiency of these formulations was examined against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory and field conditions. The laboratory evaluation of the examined formulations demonstrated that Rosa was the most potent with LC_{50} value of 0.044%, followed by Thymo, Basi and Cura since the LC_{50} values were 0.078, 0.126 and 0.192%, respectively, compared with 0.064% of Nimbecidine. The most promising formulations Rosa and Thymo were applied in the field. Data showed that, there were no significant variations between the natural prepared formulations and Nimbecidine at all tested durations. The percent reduction of *T. absoluta* populations were 84.79 and 80.80 of Rosa and Thymo, respectively, compared with 87.37% of Nimbecidine after 7 days of treatment. Concerning the side effects of the tested compounds on tomato plants, antioxidant activities of superoxide dismutase, catalase and polyphenol oxidase enzymes along with total indole acetic acid and total phenol contents had been achieved. The findings elucidated a significant decrease of all the treated examined enzymes compared with untreated plants (control). Also, photosynthetic pigments have been studied. The treatment with the tested formulations caused an increase of chlorophyll contents with time elapsed compared with the control (infested plants).

Keywords: Essential Oils; Formulations; *Tuta absoluta*; Physico-Chemical Properties; Antioxidant Enzymes

Introduction

Tomato, *Lycopersicon esculentum* Mill is a substantial vegetative crop grown throughout the world. It is the first horticultural yield in Egypt. Tomato is salutary to human health as it is rich of minerals, vitamins, necessary amino acids and sugars [1].

In recent years tomato borer moth, *T. absoluta* commonly known as tomato leaf miner has become one of the dangerous pests [2]. It also feeds on other host plants of family Solanaceae [3,4]. It was first recorded in Western Egypt in late 2009 [5]. Early instars can burrow leaves, stems, shoots, flowers and newly emerged fruits. *T. absoluta* was arisen from South America, after its elementary reveal in eastern Spain in 2006, it quickly conquered various other European countries and prevalence throughout the Mediterranean basin, involving parts of North Africa and the Middle East where it forthwith reached damaging levels [6,7]. If no control procedures are taken, then the pest can give rise to 80 - 100% yield losses in tomato crops in latterly conquered areas and may pose a menace to both greenhouse and open-field tomato output [6].

The synthetic pesticides have been considered as the main manner to control insect pests, but the development of insect resistance to these products, the high cost, environmental pollution, toxicity to humans and adverse impacts on non-target organisms have demanded the need for evolving alternative ways to control insect pest [8-11]. Plant essential oils could be an alternative provenance for insect pest control in order to they represent an affluent source of bioactive chemicals, many of them are selective and they possess a little or no injurious action towards the non-target organisms and the environment. Most of them are safe as they are commonly used as flavoring agents in foods [12,13].

Plant-based chemicals or phytochemicals, have been utilized for many years to control insect pests on agricultural crops for integrated pest management [14]. Essential oils have been demonstrated to have antifeedant, growth inhibitor, repellent and toxic effects against various insect pests [15,16].

Pesticides are formulated for increasing effectiveness in the field, promotes each of safety characteristics and handling qualities [17-19] mentioned that, adjuvants are utilized to raise the bioactivity of pesticides. The physical and chemical characteristics of the examined compounds could be utilized to locate the type of adjuvant used. In recent trend increasingly need safer and more proper pesticide formulations instead of the traditional solvent - based insecticides (EC) which trigger various problems to plants and environment [18,20].

To understand the biochemical changes caused by tested insects on tomato plants before and after treatments as the side effect, the activity of the antioxidant enzymes must be carried out. These enzymes represent a critical role in protecting plants contra insect attack. It has become evident that plants produce reactive oxygen intermediates (ROS) as signaling molecules to overcome insect attack [21]. Major ROS-scavenging techniques of plants involve antioxidant enzymes such as SOD, CAT and PPO [22].

The objective of the present work is to assess the impact of some new types of naturally prepared formulations from essential oils contra larvae of tomato leaf miner, *T. absoluta* in the framework of laboratory and field conditions. Also, the Physico - chemical properties of the examined formulations were investigated. In addition, the side effects of these formulations on the antioxidant enzymes and photosynthetic pigments of the treated plants were studied.

Materials and Methods

Insect

Populations of *T. absoluta* were established using larvae collected from untreated tomato fields, in El-fashn village-Bani Sweif Governorate. The stock culture was maintained in the laboratory. Leaves of tomato were used to feed the insect larvae during the experimental tests.

Preparation of the tested essential oils

The aerial parts of some medicinal and aromatic plants mentioned in table 1 were processed by hydro distillation for 4h using steam distillation Clevenger apparatus to obtain the essential oils.

Tested essential oils		Tested formulated compounds
Scientific name	Common name	
Curcuma longa	Curcuma or turmeric	Cura
Ocimum basilicum	Basil	Basi
Rosmarinus officinalis	Rosemary	Rosa
Thymus vulgaries	Thyme	Thymo

Table 1: Scientific, common names of the tested essential oils and their formulated compounds.

Types of the tested formulated compounds

Three formulation types were naturally prepared as follow:

Water based emulsion (EW)

The formulation was prepared by mixing rosemary essential oil with methyl salicylate and water in appropriate amounts of emulsifier and natural solvents (vegetable and mineral oils) according to the method described by [23] with some modifications.

Water in oil in water multiple emulsion (W/O/W)

The two compounds Thymo and Basi were naturally prepared as water in oil in water multiple emulsion (W/O/W) as follow:

Each of the two compounds was prepared by mixing thyme or basil oils with appropriate amounts of sodium salicylate and two emulsifiers (span 83 and tween 80) in water according to the method described by [24] with some modifications.

Emulsifiable concentrates (EC)

The formulation was prepared by mixing curcuma oil in appropriate amounts of two emulsifiers and natural solvents (mineral and vegetable oils).

Commercial Nimbecidine (EC)

This product containing 0.03%, active ingredient of azadirachtin (*Azadirachta indica*) manufactured by Stanes company LTD.

Physico - chemical properties of the tested formulations

Emulsion stability test

To prepare standard hard water, anhydrous calcium chloride (0.304g) and magnesium chloride hexahydrate (0.139g) are dissolved in distilled water and completed to 1L. The resultant water has a hardness of 342 mg/L calculated as calcium carbonate [25].

The emulsion stability test was carried out according to WHO specifications. Into a 250 ml beaker, 75 - 80 ml of hard water were poured. Five ml of the emulsifiable concentrate formulation was added by a pipette, while stirring with a glass rod. The beaker contents were completed to 100 ml by addition of the hard wa-ter, while the stirring was continuous. The beaker contents were poured immediately into graduated 100 ml cylinder. The cylinder was kept at 30 - 31°C for one hour and examined for any free oil or creaming separation. The volume of free oil, cream or solid matter, if any, should not override two ml.

Foam test

The emulsion stability test was carried out also to measure the foam amounts formed on the emulsion surface in the cylinder after five minutes. The foam layer should not exceed five ml for passing the test.

pH test

The test was carried out according to CIPAC specifications [25]. One g of the tested formulation was weighed and transferred to a measuring cylinder (100 ml) containing about 50 ml distilled water. The cylinder was made up to 100 ml and shook vigorously for 1 minute and then it was allowed to settle. The pH of the supernatant liquid was measured.

Storage stability test

Stability at 0°C

The test was carried out according to CIPAC specifications [25]. One hundred ml of the formulation were placed in the measuring cylinder and poured into a glass bottle. The glass bottle was stoppered tightly and cooled to 0 ± 2°C for 7 days and the three previous examinations were carried out.

Stability at elevated temperature

The method was carried out according to CIPAC specifications [25]. One hundred ml of liquid formulation was poured into a glass bottle. The glass bottle was sealed to avoid loss of volume and stored in an oven at 54 ± 2°C for 14 days. The stored samples are cooled to room temperature and the emulsion stability, foam and pH tests were carried out.

The toxicity of the tested compounds on *Tuta absoluta*

Laboratory experiment

The toxicity of the tested formulations on the 2nd larval instar of *T. absoluta* was determined by using standard leaf dipping (IRAC method No. 022). Several concentrations of each compound were prepared in water as emulsion. Three replicates of each treatment were used. Numbers of dead insects were cumulatively counted till 48h of treatment and mortality percentages were estimated. Leaves treated with water only used as a control. LC_{50}, LC_{90} and slope values were calculated as described by [26].

Field Evaluation

Field experiments were carried out on infested tomato plants with *T. absoluta* cultivated in Elfashn village - Bani Sweif Governorate. A known area was divided into plots, each one is $42m^2$. Tomato plants were sprayed with the most efficient formulations, Rosa and Thymo at LC_{90} values multiplied by 3, in addition, the recommended dose of the commercial formulation, Nimbecidine.

Samples of treated plants were taken after periods of (0, 1, 3, 5 and 7 days of treatment). Other samples of non-treated infested plants were also taken after the same previously periods and used as a control. The reduction percent of infestations was calculated according to Henderson-Tilton formula [27].

The side effects of the tested compounds on treated tomato plants

Enzymatic and non-enzymatic antioxidants

The activity of the enzymatic and non-enzymatic antioxidants of the tomato treated plants were evaluated at leave samples which collected at the previously mentioned periods as follow:

Plant tissue preparation

Enzyme extracts were prepared according to the method described by [28]. Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8,000 rpm and 4°C for 15 minutes. The supernatant was used immediately to determine the activities of the tested enzymes.

Super oxide dismutase (SOD)

SOD (EC 1.12.1.1) activity was spectro-photometrically assayed at 560 nm by nitro-blue-tetrazolium (NBT) reduction method [28].

Catalase (CAT)

CAT (EC 1.11.1.6) activity was determined spectro-photometrically by following the decrease in absorbance at 240 nm [29].

Poly phenol oxidase (PPO)

PPO (EC 1.10.3.1) activity was determined using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm [30]. The absorbance at 410 nm was recorded continuously at 25°C for 5 minutes.

Indole acetic acid (IAA)

A known weight of the fresh samples of leaves was taken and extracted with 85% cold methanol (v/v) for three times at 0°C. The combined extracts were collected, dried under vacuum and made up to a known volume with distilled water. One ml of this water extract and 4 ml of para - dimethyl amino benzoic acid reagent, which contain 1g dissolved in 50 ml HCl and 50 ml of ethanol 95%, were left for 60 minutes in 30 - 40°C. The developing colour was spec-trophotometrically measured at 530 nm [31].

Total phenolic contents

The fresh leaves were extracted as IAA extraction, and then 0.5 ml extract was added to 0.5 ml Folin reagent, shaken and allowed to stand for 3 minutes. Then, 1 ml of saturated sodium carbonate was added to each tube, followed by distilled water, shaken and allowed to stand for 60 minutes. The optimal density was determined at 725 nm using spectrophotometer [32].

Photosynthetic pigments content

Chlorophyll a, chlorophyll b and total carotenoids contents were extracted from one gram of longitudinal sections of fresh leaves and ground in a mortar in 85% acetone. The optical density (O.D.) of the solution was recorded at 663, 644 and 452.5 nm for chlorophyll a, b and carotenoids, respectively measuring spectro-photometrically according to [33] and their levels were calculated according to the formula of [34]. Values of photosynthetic pigments were expressed in mg/g fresh weight.

Results and Discussion

Physico-chemical properties of the tested formulations

The findings of emulsion stability, foam formation and pH of the tested green based formulations in addition to Nimbecidine commercial formulation are revealed in table 2. All the tested formulations succeed the emulsion stability, since; Rosa did not record any separation layers, whether, before, after cold or heat storage. Anyway, the highest separation value of the green - based formulations did not exceed 0.5 ml at any of aforementioned emulsion stability steps. The emulsion stability of Nimbecidine commercial formulation were 0.5, 1 and 0.5 ml before storage, after cold and heat storage, respectively (The volume of cream layer, if any, should not exceed two ml).

Formulation	Emulsion stability (separation ml)			Foam formation (ml)			pH		
	Before storage	After cold storage	After heat storage	Before storage	After cold storage	After heat storage	Before storage	After cold storage	After heat storage
Basi	0.5	0.5	0	0.3	0	0.3	7	7	7
Cura	0	0.5	0	0.8	0.8	0.5	6.5	6.6	6.6
Rosa	0	0	0	0	0	0	7.2	7.1	7
Thymo	0.5	0.5	0.5	2.5	1	0.5	7.3	7.3	7.2
Nimbecidine	0.5	1	0.5	0	0	0	7.1	7.2	7.1

Table 2: Physico-chemical properties of the tested formulations.

Concerning the foam formation, the prepared and commercial formulations passed it. Each of Rosa and Nimbecidine did not exhibit any foam formations. The foam formations of Basi and Cura ranged from 0.3 - 0.8 ml. Thymo recorded the highest foam layer before storage with value of 2.5 ml (the limit of foam layer volume should not exceed 5 ml) [25]. This implies that, the examined formulations could be applied in the field without any foam problems.

The pH of all tested formulations was around seven, except Cura, (pH equal 6.5 before storage and 6.6 after cold and heat storage) [35] reported that most pesticides are most stable when the spray solution between pH 5.5 - 7.0.

The insecticidal efficiency of the tested compounds against 2nd instar larvae of *Tuta absoluta*

Laboratory Experiment

The LC_{50} and LC_{90} (%) values, 95% confidence limits and other regression analysis parameters of the tested green based formulations along with Nimbecidine contra the 2nd instar larvae of *T. absoluta* are given in table 3. Rosa was the most superior with LC_{50} value of 0.044%, followed by Nimbecidine and Thymo with LC_{50} values of 0.064 and 0.078%, respectively. The corresponding values of LC_{90} were 0.38, 0.571 and 0.187%, respectively. Moreover, Basi and Cura showed toxicity with LC_{50} values of 0.126 and 0.192%, respectively, furthermore, the corresponding values of LC_{90} were 0.540 and 1.04%, respectively. The impact of essential oils in controlling *T. absoluta* was demonstrated in laboratory tests [36,37].

Formulations	LC_{50}[a] (%)	95% Confidence limits (%)		LC_{90} (%)	95% Confidence limits (%)		Slop ± (SE)[b]	Intercept ± (SE)[c]	(χ^2)[d]
		Lower limit	Upper limit		Lower limit	Upper limit			
Basi	0.126	0.117	0.137	0.540	0. 451	0.678	2.03 ± 0.13	1.82 ± 0.12	15.51
Cura	0.192	0.131	0.250	1.04	0.709	2.089	1.75 ± 0.12	1.25 ± 0.07	48.26
Rosa	0.044	0.081	0.12	0.384	0.286	0.604	2.19 ± 0.11	1.51 ± 0.08	66.10
Thymo	0.078	0.063	0.094	0.187	0.141	0.337	3.35 ± 0.21	3.72 ± 0.23	81.63
Nimbecidine	0.064	0.031	0.097	0.571	0.425	0.919	1.35 ± 0.22	1.61 ± 0.08	37.52

Table 3: Toxicity of the tested essential oil formulations against 2nd instar larvae of *Tuta absoluta* using leaf dipping technique.

[a]: Concentration causing 50% mortality after 24 h. of treatment; [b]: Slope of concentration mortality regression line; [c]: Intercept of regression line; [d]: Chi square value.

Field Evaluation

Reduction percentages of *T. absoluta* infestation applied with the most potent natural based formulations, Rosa and Thymo, as well as Nimbecidine are exhibited in table 4. The results indicated that, all tested insecticides reduced the population of *T. absoluta* infestation. Rosa, Thymo and Nimbecidine were not significantly different at all examined intervals (0, 1, 3, 5 and 7 days), since, the percent reduction of infestation after 7 days from application were 84.79, 80.80 and 87.37 respectively.

Compound	Before-spray (Alive)	Reduction (%) at indicated durations after application (days)									
		0		1		3		5		7	
		P	%R	P	%R	P	%R	P	%R	P	%R
Rosa	23.33	22.00	17.03	10.33	62.04[a*]	6.33	74.77[a]	5.67	79.45[a]	3.33	84.79[a]
Thymo	20.33	19.33	16.33	8.33	64.87[a]	4.67	78.67[a]	5.00	79.19[a]	3.67	80.80[a]
Nimbecidine	19.67	18.67	16.47	8.33	63.68[a]	4.67	77.94[a]	4.00	82.79[a]	2.33	87.37[a]
Control	22.00	25.00	0	25.67	0[b]	23.67	0[b]	26.00	0[b]	20.67	0[b]
$LSD_{0.05}$			N.S		23.2		18.73		15.91		8.91

Table 4: Reduction percentages in infestation of *Tuta absoluta* larvae treated with the tested compounds.
P = No. of population; %R = Reduction percentages of infestation; $L.S.D_{0.05}$ least significant difference at 0.05 level of probability; N.S not significant difference at 0.05 level of probability; *Mean followed by the same letter in a column are not significantly different at 5% level of probability (Duncan Test).

The reduction averages ranged from 62.04 to 64.87% after one day from the application and ranged from 74.77 to 78.67, 79.19 to 82.79 and 80.80 to 87.37% after 3, 5 and 7 days from application, respectively.

Various derivatives of vegetable source, particularly the form of essential oils, have been proposed as replacements to synthetic pesticides [38]. There are many authors assessed the efficacy of essential oils contra *T. absoluta* and gained satisfying results [36,39].

The side effects of the tested formulations

To explain the biochemical changes induced by *T. absoluta* in tomato plants before and after treatments, enzyme and non-enzyme antioxidants along with the photosynthetic pigments would be determined.

Antioxidant Enzymes

Super oxide dismutase (SOD)

Data presented in table 5 showed that all the examined formulations significantly decreased the activity of SOD enzyme compared with control (infested plants) at all different tested intervals. In addition, all tested compounds were not significantly different in their activity, except after 7 days, since, the activities of Rosa and Nimbecidine were not significantly different (401 and 394 U/g fresh weight/h), respectively. These findings confirm the results of field evaluation where these two compounds had the highest efficiency.

Enzyme activity (U/g fresh weight/h)	Treatment	Days after treatment			
		1	3	5	7
S.O.D	Rosa	491.0 ± 32.5[b*]	470.0 ± 19.8[b]	450.0 ± 28.3[b]	401.0 ± 9.90[c]
	Thymo	497.0 ± 18.4[b]	480.0 ± 50.9[b]	455.0 ± 9.90[b]	439.0 ± 15.6[b]
	Nimbecidine	489.0 ± 18.4[b]	474.0 ± 39.6[b]	443.0 ± 21.2[b]	394.0 ± 5.70[c]
	Control	554.0 ± 17.6[a]	562.0 ± 42.5[a]	582.0 ± 32.3[a]	588.0 ± 22.7[a]
	L.S.D	29.34	24.76	20.49	30.56
Catalase	Rosa	28.85 ± 0.59[b]	26.13 ± 3.08[b]	24.50 ± 3.84[b]	23.73 ± 6.93[b]
	Thymo	31.03 ± 3.44[b]	28.31 ± 1.54[b]	25.59 ± 0.77[b]	25.60 ± 3.85[b]
	Nimbecidine	30.49 ± 1.54[b]	26.68 ± 0.77[b]	22.32 ± 5.39[b]	21.78 ± 6.16[b]
	Control	40.83 ± 0.77[a]	37.56 ± 0.77[a]	34.84 ± 3.08[a]	39.20 ± 1.54[a]
	L.S.D	6.29	4.16	4.20	5.64
Polyphenol oxidase	Rosa	41.20 ± 0.1[b]	27.36 ± 3.80[b]	24.88 ± 5.8[b]	18.40 ± 5.7[b]
	Thymo	39.44 ± 1.5[b]	27.92 ± 0.60[b]	24.64 ± 0.2[b]	18.56 ± 5.9[b]
	Nimbecidine	39.84 ± 2.0[b]	29.76 ± 11.3[b]	22.56 ± 4.8[b]	16.48 ± 2.3[b]
	Control	51.04 ± 1.6[a]	68.64 ± 1.10[a]	71.36 ± 5.7[a]	71.76 ± 7.8[a]
	L.S.D	3.22	3.95	6.51	10.45

Table 5: Effect of the tested compounds on some oxidative enzymes of tomato plants.

Each value represents the mean of three replicates ± standard error; *Mean with different letters in a column were significantly different at 0.05 level of probability (Duncan Test).

Catalase (CAT)

Based on the effect of the examined compounds on CAT (table 5), the same trend of activity was obtained as for SOD, where the activities of these natural compounds and commercial insecticide were not significantly different compared with control at all tested intervals. For example, the activity of catalase on Rosa, Thymo and Nimbecidine after 7 days were 23.73, 25.60 and 21.78 U/g fresh weight/h, respectively, compared with the control of 39.20 U/g fresh weight/h. The antioxidant enzymes were reduced progressively with time elapsed compared with control (infested plants) after application with the tested formulations.

The antioxidant system, SOD and CAT work as supplementary enzymes and play a pivotal turn as a protective factor in detoxifying the reactive oxidant species [40]. SOD, in both plants and animals, is recognized for its function in destroying superoxide (convert $O_2^{\cdot-}$ to H_2O_2), while H_2O_2 is disposed by the action of CATs [41]. The antioxidant enzymes act almost immediately after the stress; and when the active oxygen form are under control, the amount of antioxidant enzymes decreased [42].

It is well known that the insect infestation increased plant enzymes activity to defend them against insect offensive, thus, the results elucidate a significant decrease of all the treated examined enzymes [43]. Sundry authors have stated that plant extracts (azadirachtin) affect several antioxidant enzymatic activities in various tissues where cells are generating enzymes [44].

Poly Phenol Oxidase (PPO)

Data given in table 5 clarified that the activity of PPO varied according to the *T. absoluta* infestation level. Generally, the infestation induced significant increase in PPO activity. All the tested formulations were not significantly different in their activity at all tested intervals. The activity of PPO after 7 days on Rosa, Thymo and Nimbecidine were 18.40, 18.56 and 16.48 U/g fresh weight/h, respectively, compared with the control of 71.76 U/g fresh weight/h. Moreover, the activity of PPO on Rosa, Thymo and Nimbecidine ranged from 39.44 to 41.20, 27.36 to 29.76 and 22.56 to 24.88 U/g fresh weight/h after 1, 3 and 5 days of treatment, respectively, compared with the control of 51.04, 68.64 and 71.36 U/g fresh weight/h, respectively.

Non-enzyme antioxidant

Total indole acetic acid (IAA)

Results in table 6 showed a reduction in the IAA content in the untreated tomato plants (control) compared to plants treated with the tested formulations. Rosa and Thymo formulations showed the highest efficiency for increasing the total IAA content after 1day (273.89 and 277.78) and after 3 days of treatment (279.44 and 282.78) mg/g fresh weight, respectively, which ascribed to reduce the insect infestation. It is worth mentioning that the tested natural formulations were more efficient than the commercial insecticide Nimbecidine as IAA increased, while after 5 and 7 days Nimbecidine was equal to Thymo in their efficiency.

Treatment	Total indole (mg/g fresh weight) at indicated Days after treatment			
	1	3	5	7
Rosa	273.89 ± 27.5[a*]	279.44 ± 13.4[a]	279.44 ± 27.5[b]	287.78 ± 2.60[b]
Thymo	277.78 ± 18.9[a]	282.78 ± 13.4[a]	286.67 ± 9.40[a]	308.33 ± 7.10[a]
Nimbecidine	261.11 ± 14.1[b]	271.67 ± 16.5[b]	297.22 ± 10.2[a]	303.89 ± 18.1[a]
Control	177.22 ± 0.8[c]	180.56 ± 16.5[c]	186.11 ± 22.8[c]	177.78 ± 4.70[c]
$LSD_{0.05}$	11.34	7.69	9.75	5.88

Table 6: Effect of the tested compounds on total indole of tomato plants.

Each value represents the mean of three replicates ± standard error; *Mean with different letters in a column were significantly different at 0.05 level of probability (Duncan Test).

IAA is the main plant growth hormone and is participated in the regulation of nearly every stage of plant development [45]. IAA stimulates stem elongation, cell expansion and growth rate, protein content and growing photosynthetic activities in flora [46]. IAA induces root growth resulting in enhancement of water and mineral uptake performance, hence, a significant increase in plant growth percentage [47,48]. Our results are in agreement with [43] who observed a reduction in the total IAA content in the untreated plants compared to plants applied with the essential oil based formulations.

Total phenol contents

The results in table 7 indicated that the total phenol contents in tomato plants treated with the examined formulations ranged from 67.66 to 93.81 compared to control (121.02 to 126.14) mg/g fresh weight. The increase in total phenol of control was due to the increase of infestation with insects. Moreover, the total phenol of plants applied with the tested compounds was decreased as results of reduction in infestation and they were not significantly different at all tested intervals.

Treatment	Total phenol (mg/g fresh weight) at indicated days after treatment			
	1	3	5	7
Rosa	93.81 ± 5.20^{b*}	81.97 ± 8.50^{b}	72.79 ± 2.5^{b}	69.25 ± 1.3^{b}
Thymo	91.16 ± 6.50^{b}	78.26 ± 4.70^{b}	71.20 ± 2.2^{b}	70.32 ± 3.2^{b}
Nimbecidine	86.57 ± 12.3^{b}	79.50 ± 5.50^{b}	71.20 ± 4.7^{b}	67.66 ± 2.2^{b}
Control	124.20 ± 11.7^{a}	126.14 ± 2.5^{a}	121.02 ± 4.2^{a}	121.19 ± 9.2^{a}
$LSD_{0.05}$	7.83	4.37	4.65	4.19

Table 7: Effect of the tested compounds on total phenol contents of tomato plants.

Each value represents the mean of three replicates ± standard error; *Mean with different letters in a column were significantly different at 0.05 level of probability (Duncan Test).

PPO enzyme possibly used in the defense reaction and hypersensitivity involving resistance of plant to biotic and abiotic stresses [49]. Moreover, total phenols probably work as substrates of the antioxidant enzyme; for that, the phenolic content of the infested plants was accompanied by the percent of increase in PPO that oxidise phenolic compounds to quinines [50].

Photosynthetic pigments

Regarding photosynthetic pigments of tomato plant treated with the tested compounds after one hour, 1, 3, 5 and 7 days of treatment, the results recorded in figures 1, 2, 3 and 4 showed that after one-hour chlorophyll (a), (b) and carotenoids had no significant differences between the treated and untreated plants. On the other hand, chlorophyll (a) and carotenoids contents were significantly superior in the treated plants (non-infested) than the control (infested plants) after 1, 3, 5 and 7 days of treatment. Generally, the total pigment contents were higher of treated plants than untreated plants. It could be concluded that insect infestation had a negative impact on the chlorophyll and carotenoid contents.

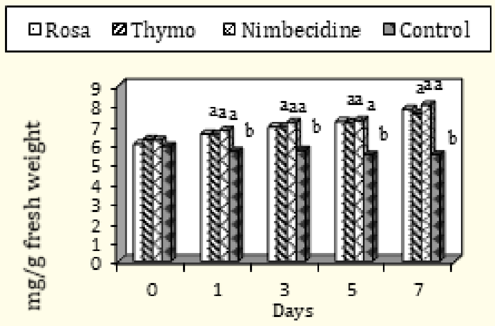

Figure 1: Effect of the tested compounds on chlorophylla of tomato plant. The graph bars with no letters were non-significant. The bars containing similar letters are not significantly different (p = 0.05).

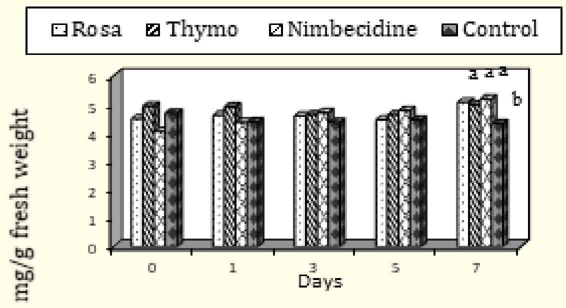

Figure 2: Effect of the tested compounds on chlorophyllb of tomato plant. The graph bars with no letters were non-significant. The bars containing similar letters are not significantly different (p = 0.05).

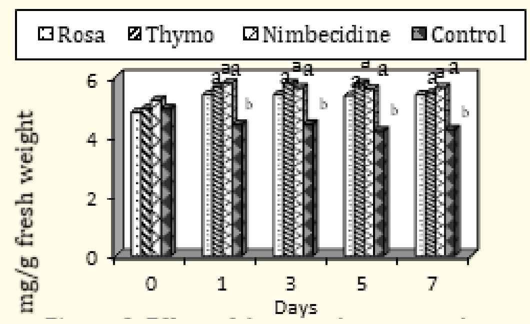

Figure 3: Effect of the tested compounds on carotenoids of tomato plant. The graph bars with no letters were non-significant. The bars containing similar letters are not significantly different (p = 0.05).

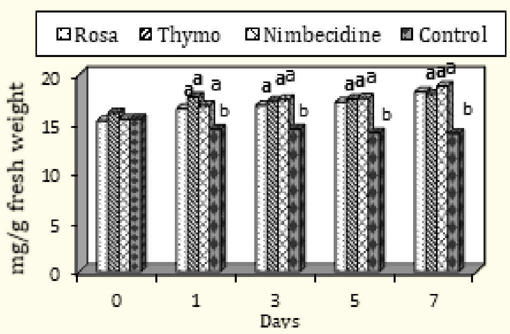

Figure 4: Effect of the tested compounds on total piggments of tomato plant. The graph bars with no letters were non-significant. The bars containing similar letters are not significantly different (p = 0.05).

Gomez SK., *et al.* [51] reported that the feeding of herbivorous insects occurs biochemical and physiological changes in the host plants, influencing the life operations of plants like photosynthesis. Golan K., *et al.* [52], Goławska S., *et al.* [53] mentioned that the chlorophyll levels change through plant growth, and nutritional defect occur as a result of a wide diversity of stresses comprising biotic stresses such as insect feeding. Moreover, Huang TI., *et al.*[54] stated that relative chlorophyll loss was relevant to the level of feeding damage induced by the insect [55] mentioned that *Bemisia tabaci* infested tomato plants led to decrease of the leaf photosynthesis by diminishing the level and photosynthetic capacity of chlorophyll.

Conclusion

The present study is useful for evolving alternative ways to control insect pest and avoid their hazards. So, using theses green based essential oil formulations (Basi, Cura, Rosa and Thymo) are promising tools for controlling *T. absoluta* instead of conventional insecticides.

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