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Indirect Effects of Zinc Oxide Nanoparticles and Grafting Technique on the Performance of the Two-Spotted Spider Mite Populations and the Growth of Greenhouse-Grown Cucumbers

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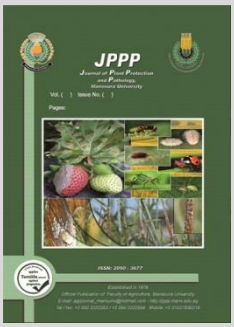

ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) have garnered significant interest due to their role in enhancing seed germination, plant growth, seedling vigor, and crop pests' management. The present study assessed the indirect effects of ZnO-NPs foliar application (as a supplementary nanofertilizer) and grafting technique on the suitability of cucumber plants for the two-spotted spider mite, *Tetranychus urticae* Koch (TSSM) and its natural predators in greenhouses. The effects on cucumber's vegetative growth, biochemical parameters, and yield were also evaluated. The plant leaves were treated with ZnO-NPs at four concentrations: 0 (control), 100, 200, and 300 mg L^{-1} in three rounds. The efficacy of ZnO-NPs in suppressing the density of TSSM eggs and motile stages was dependent on concentration. The recent findings indicated that grafted plants subjected to 200 and 300 mg L^{-1} exhibited the lowest counts of TSSM eggs and motile stages in comparison to non-grafted plants. The application of ZnO-NPs also increased the population density of both phytoseiid predators *Phytoseiulus persimilis* A.-H and *Neoseiulus cucumeris* (Oudemans). Results also showed that the interaction between grafting and ZnO-NPs application significantly enhanced plant length, leaf dry matter, photosynthetic pigment content, and fruit yield. The leaf concentrations of macro- and micro-nutrients, along with biochemical leaf parameters, were markedly improved. These findings demonstrated the efficacy of ZnO-NPs and grafting technique as a combined treatment in suppressing TSSM density, augmenting the effectiveness of natural predators, and simultaneously improving cucumber growth and yield.

Keywords: *Tetranychus urticae*, *Phytoseiulus persimilis*, *Neoseiulus cucumeris*, ZnO nanoparticles, grafting, macronutrients, phenols

INTRODUCTION

Plants' nutritional composition might be positively associated with their suitability to herbivorous pests as an aspect of resistance mechanisms against herbivorous pests (Chaboussou, 2004; Joern *et al*., 2012). Consequently, it may be feasible to indirectly regulate and diminish pest populations by altering crops' elemental composition (Altieri and Nicholls, 2003; Huber *et al*., 2012). Nevertheless, the extent to which zinc oxide nanoparticles (ZnO-NPs) can modify plant elemental composition or enhance defensive characteristics to diminish crop suitability to herbivorous pests remains unknown. Furthermore, the suitability of a particular host plant may correlate with its elemental composition (Nansen *et al*., 2021).

Nutrient management might offer a novel approach to spider mite control. Providing plants with essential nutrients during crucial growth phases (phenological stages) may bolster their innate defenses. This improvement may entail strengthening plant structures and boosting metabolic processes. Ultimately, these changes could lead to a more potent antibiosis effect, making plants more resistant to a broader range of mites (Abou Zaid *et al*., 2018; Taha *et al*., 2019). Chávez-Dulanto *et al*. (2018) demonstrated that the foliar application of nutrients, including macro-elements such as calcium and magnesium as well as micro-elements like iron (Fe), manganese (Mn), zinc (Zn) and boron, effectively regulated the populations of the citrus red mite *Panonychus citri* McGregor (Acari: Tetranychidae) and the citrus rust mite *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae).

The hypothesis that supplementing plants with ZnO-NPs and/or using a grafting technique might indirectly affect the host plant's suitability to the two-spotted spider mite (TSSM) *Tetranychus urticae* Koch (Acari: Tetranychidae). The present study tested the following hypotheses: (1) supplemental ZnO-NPs induce significant alterations in the biochemical components of cucumber leaves including phenols, proteins, photosynthetic pigments, and carbohydrates, as well as the content of macro-elements [Nitrogen (N), Phosphorus (P), and Potassium (K)] and micro-elements (Zn), (2) supplemental ZnO-NPs enhance plant growth and crop yield, (3) the population density of TSSM will be suppressed in plants treated with ZnO-NPs and/or grafted plants, and (4) supplemental ZnO-NPs may affect TSSM egg-laying capacity (fecundity) and may promote an increase in predatory mite populations on ZnO NPs -treated and/or grafted plants.

To test these hypotheses, the TSSM was selected as a destructive pest of cucumbers, characterized by its rapid life cycle and substantial reproductive potential (Park and Lee, 2005; Tehri *et al*., 2014). Synthetic miticides remain the dominant approach for managing this pest; however, alternative strategies are necessary to mitigate environmental impacts and resistance issues (Attia *et al*., 2013). Global

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alternative control measures include the use of natural predators, such as *Phytoseiulus persimilis* (Acari: Phytoseiidae) (McMurtry *et al*., 2013). Despite their potential, the adoption of biological control programs in commercial cucumber production is constrained by inconsistent effectiveness and the increased burden of monitoring pest populations (Tiftikçi *et al*., 2022).

The grafting technique presents a promising alternative strategy that provides a compelling solution along with supplementary advantages. Grafting enhances plant growth and development while strengthening natural defenses against biotic stresses, leading to improved fruit yield and quality under adverse conditions (Louws *et al*., 2010; Shoorooei *et al*., 2013). Numerous studies indicated that the grafting technique effectively suppressed the density of spider mites and other insect pests, serving as a valuable component when integrated with other strategies of Integrated Pest Management (IPM) (Edelstein *et al*., 2000; Álvarez-Hernández *et al*., 2009; Žanić *et al*., 2017; Ismail and Hussien, 2024).

While these practices demonstrate some effectiveness, they may not consistently ensure adequate control of the TSSM population, especially in greenhouses. Therefore, supplementary management strategies are required to augment these practices and improve their efficacy. Therefore, the present work aims to use nano-structured zinc as an additional application may align well with other IPM tactics, serving as an innovative and sustainable element in contemporary pest management.

MATERIALS AND METHODS

The trial was conducted on a private farm in the 10th of Ramadan Agricultural Cooperative Society (30°39'55.8"N 32°10'24.2"E), Ismailia Governorate, Egypt, under greenhouse conditions from January to May 2024.

Host plant growing conditions and grafting method

The cucumber (*Cucumis sativus* L.) cv. "Barracuda F1" (Seminis Vegetable Seeds Company, USA) grafted onto a hybrid rootstock "Nun 6001" (*Cucurbita maxima* × *C. moschata*) (Nunhems Seed Company, USA) was used. The scion seeds were sown in seedlings trays (216 cells) 3 to 5 days prior to the sowing of the rootstock seed. The splice graft (one cotyledon) technique was employed for grafting as previously described by Lee *et al*. (2010). Rootstock and scion seedlings must possess a minimum of one true leaf. The rootstock was precisely excised with a sharp blade at an angle to eliminate one cotyledon and the growing point. The angle must approximate 45 degrees to optimize surface area. The hypocotyl of the scion was severed at an angle of 35 to 45 degrees on one side only. The scion and rootstock were secured with a grafting clip to ensure proper alignment and union. The grafted seedlings were positioned within a plastic tunnel in the healing chamber, maintained at 25-30°C and relative humidity exceeding 85%.

The seedlings (30 days old) were transplanted in the first week of January, with a spacing of 70 cm × 30 cm (row x plant). The crop was cultivated in a pesticide-free setting, adhering to established agronomic practices. All plants were administered the prescribed dosage of NPK fertilizers. Nitrogen was applied at a rate of 110 kg per feddan, utilizing ammonium nitrate with a nitrogen content of 33.5%. Potassium sulfate (48% K_2O) and Calcium superphosphate (15.5% P_2O_5) were applied at rates of 90 kg and 40 kg/feddan, respectively.

Experimental design and ZnO-NPs application

The powdered ZnO-NPs (purity 99.5%) were sourced from Sigma Aldrich (St. Louis-USA). The experiment utilized a randomized complete block design and involved eight treatments. These treatments consisted of grafted and non-grafted plants sprayed with three varying ZnO-NPs: 100, 200, and 300 mg L^{-1} concentrations, in addition to a control group, which were sprayed with water. Each treatment was replicated three times. Three rounds of foliar applications were conducted utilizing a 20 L backpack sprayer. The initial ZnO-NPs application occurred 10 days following the grafting process. The second spraying was administered 10 days after transplanting, while the third application took place 15 days after the second spray.

Assessment of two-spotted spider mite populations

The incidence of TSSM occurred naturally, and evaluations began upon the observation of yellow punctuations on leaves. Every ten days, thirty-six leaves were randomly plucked per treatment, and the number of eggs and motile stages of TSSM was quantified in a 2.5 cm² area on the leaf's underside. Predators were also enumerated across the entire leaf surface using a stereo-zoom binocular microscope. The population density of TSSM was quantified as the cumulative number of mites per day (CMD), determined by averaging the mite count per plant and multiplying by the number of intervening days. The percentage of infested leaves (prevalence) was also calculated.

Estimation of mineral elements content in cucumber leaves

The macro- (N, P, K) and micro- (Zn) element contents were measured in grafted and non-grafted cucumber leaves treated with ZnO-NPs. Leaf samples collection occurred 60 days after the third spray. The leaves were dried at 70°C for 72 hrs. Subsequently, the dried leaves were digested using H_2So_4 and H_2O_2 according to the procedure described by Jackson (1967). The resulting extract was obtained for elemental determination. Nitrogen and potassium contents were determined according to the procedure of Jackson (1967). Nitrogen was measured using the Kjeldahl method, while potassium was quantified by flame photometry (Jenway PFP7 Flame Photometer, UK). The phosphorus content was determined spectrophotometerically (Jenway spectrophotometer, UV/Vis, UK) following the method outlined by Black *et al*. (1965). Zinc content was assessed following wet digestion utilizing a nitric (HNO_3)-sulfuric (H_2SO_4) perchloric ($HClO_4$) acid mixture (4:1:8 v/v) following the method outlined by Jackson (1973). The concentration was measured utilizing flame atomic absorption spectrometry (Thermo Scientific S Series-GE 711838-Thermo Electron Corporation-Waltham-USA).

Biochemical analyses

Biochemical analyses were conducted on oven-dried cucumber leaves obtained from ten distinct plants subjected to ZnO-NPs treatment. All experiments were performed in triplicate.

Total phenol content

The extraction assay was conducted according to the methodology outlined by Kähkönen *et al*. (1999). The Folin–Ciocalteu method (Singleton and Rossi, 1965) was employed for quantification, utilizing gallic acid as the standard (mg GAE

g^{-1} DW). Absorbance was calculated spectrophotometrically at 765 nm (Milton Roy Spectronic 1201, UV/Vis spectrophotometer, USA).

Total protein content

The extraction assay was conducted according to the methodology outlined by Hildebrand *et al.* (1986). The total protein content was assessed using the dye-binding method (Bradford, 1976), with bovine serum albumin serving as the standard. Absorbance was estimated at 595 nm (Milton Roy Spectronic 1201, UV/Vis spectrophotometer, USA).

Chlorophyll and carotenoid contents

Chlorophyll (Chl.) a, b, and carotenoid contents were measured according to Lichtenthaler (1987). Fresh leaves (500 mg) were extracted overnight with 80% acetone, subsequently undergoing centrifugation for 5 min at 10,000 rpm. Observance of Chl. a, b, and carotenoids were detected spectrophotometrically (Unico spectrophotometer, UV/Vis, USA) at OD647, 663, and 470.

Vegetative growth and crop yield

The measurements of plant length and leaves dry matter percentage (LDM%) were recorded 60 days after the third spray. The length of the main stem was measured using a steel measuring tape. The LDM% was assessed by weighing 50 leaves from ten plants per replicate, followed by drying in an oven at 70°C for three days to measure dry weight. The LDM% was determined using the formula: leaf dry weight (g) / leaf fresh weight (g) x 100.

During each harvest, the weight of all fruits, both marketable and non-marketable, from ten plants per replicate were quantified and measured.

Statistical analysis

Tests were performed to validate the assumptions required for the analysis of variance (ANOVA) to evaluate the homogeneity of variances and the normality of data distribution. A two-way ANOVA analysis was subsequently conducted on all parameters. The Duncan Multiple Range test ($P \leq 0.05$) was utilized to identify significant differences between means.

RESULTS AND DISCUSSION

The two-spotted spider mite and its associated predatory mite's performance

Grafted plants consistently demonstrated significantly reduced counts of motile and egg stages compared to non-grafted plants across all measured days after spraying (DAS), with the exception of 10 DAS (Table 1 and 2). Grafted plants exhibited significantly lower cumulative mite days (CMD) and prevalence compared to non-grafted plants (Table 3). This is consistent with the research conducted by Ismail and Hussien (2024), which demonstrated that grafting markedly decreased the density of TSSM on eggplants through the enhancement of antioxidant enzyme activities. This technique has effectively suppressed additional insect pests in cucurbits and solanaceous crops. Grafting tomatoes onto wild relatives led to a 1.7 to 3-fold reduction in aphids and whiteflies incidence compared to non-grafted plants (Álvarez-Hernández *et al*., 2009). Similarly, Žanić *et al*. (2017) demonstrated that grafting tomatoes onto specific rootstocks reduces whiteflies density. Furthermore, modifications in leaf anatomy due to rootstocks were correlated with a reduction in whiteflies population density, as indicated by Žanić *et al*. (2018). Grafting cucumber plants onto particular rootstocks can enhance the plants' defenses against TSSM infestations by affecting the production and transport of defensive phytochemicals, such as cucurbitacin-C, which are toxic to spider mites and serve as repellents (Agrawal *et al*., 1999; Balkema-Boomstra *et al*., 2003).

The findings of this study clearly indicated that the foliar application of ZnO-NPs had a significant effect on the counts of TSSM motile and egg stages (Table 1 and 2). The plants treated with 200 and 300 mg L^{-1} exhibited lower counts of the motile stage compared to those treated with 100 mg L^{-1} and the control group (untreated plants). The egg counts indicate the reproductive capacity of TSSM. The control group exhibited the highest egg counts at all DAS, reaching the peak at 70 DAS. In contrast, plants treated with ZnO-NPs at concentrations of 100, 200, and 300 mg L^{-1} demonstrated reductions in egg counts of 43.24%, 71.47%, and 66.43%, respectively, compared to the control group. The concentrations of 200 and 300 mg L^{-1} ZnO-NPs were more effective than 100 mg L^{-1} in reducing egg numbers, indicating a suppression of TSSM fecundity. The control group demonstrated the highest CMD, exceeding treated plants by 70.50%, 70.51%, and 48.18% for concentrations of 300, 200, and 100 mg L^{-1}, respectively. The control group exhibited the highest prevalence of TSSM (75 ± 4.06%), whereas the lowest prevalence was noted in plants treated with 200 mg L^{-1} (46.76 ± 2.99%) as shown in Table (3).

The present results hypothesized that supplementing cucumber plants with ZnO-NPs and/or using a grafting technique may influence the host plants, thereby affecting the population dynamics of the TSSM. The combined effect of grafting and foliar application of ZnO-NPs significantly affected the counts of motile and egg stages of TSSM at all measured intervals, with the exception of 10 DAS. The grafted plants subjected to 300 mg L^{-1} exhibited the lowest counts of motile stages in comparison to other treatments. The counts reached a peak at 90 days DAS, averaging 6.17 ± 0.30 individuals per leaf, which represents reductions of 68.36% and 79.60% compared to the grafted and non-grafted control groups, respectively. The motile stages of mite in non-grafted plants treated with 200 and 300 mg L^{-1} peaked at 90 days reached 12.08 ± 0.65 and 13 ± 0.72 individuals per leaf, respectively. During the study period, the lowest egg counts were recorded in grafted plants treated with 200 and 300 mg L^{-1}, with counts ranging from 0.0 ± 0.0 to 13.83 ± 1.16 and 0.08 ± 0.08 to 12.67 ± 0.94 eggs per leaf, respectively. Additionally, grafted plants which subjected to 200 mg L^{-1}, also exhibited the lowest CMD (828.33 ± 35.45). The value was roughly 33.37% lower than the CMD recorded in the non-grafted plants subjected to the same concentration. A comparable trend was noted for grafted plants subjected to 300 mg L^{-1} treatment, exhibiting a 28.84% reduction in CMD compared to non-grafted counterparts as shown in Table (3).

These findings align with those obtained by Chávez-Dulanto *et al.* (2018) who demonstrated that the foliar application of microelements, particularly Zn, serves as a viable alternative method for managing citrus red spider mites. Similarly, Abou Zaid *et al.* (2018) demonstrated that foliar applications of Zn, Mn, and Fe effectively managed TSSM populations on beans. Furthermore, the application of micronutrients in hot peppers markedly decreased the population density of TSSM as well as whiteflies (Shafeek *et al*., 2014).

The suppression is linked to the presence of Zn in the host plant's leaves, which may have modified the nutritional quality or palatability of the plant material, rendering it less suitable as a food source for the TSSM. The presence of ZnO-NPs on the leaf surface may serve as a physical and/or chemical deterrent, inhibiting TSSM from settling and feeding on the plant. This would adversely affect the survival, fecundity, and population growth of the TSSM. Research conducted by Rasim *et al*. (2021), Puspitarini *et al*. (2021), and Senbill *et al*. (2023) have shown that the incorporation of ZnO-NPs in host plants markedly decreases the survival, fecundity, and population growth of the TSSM.

During the studied season, two predatory phytoseiid mites were detected; *Phytoseiulus persimilis* and *Neoseiulus cucumeris*. The results showed that the non-grafted plants exhibited a significant increase in *P. persimilis* count. In contrast, the grafted plants exhibited a marginal increase in *N. cucumeris*, with no significant difference compared to the non-grafted plants (Table 3).

Table 1. The influence of foliar application of ZnO-NPs and grafting technique on the motile stage counts of the two-spotted spider mite in cucumber.

ZnO-NPs (mg L^{-1})	10 DAS*	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS	90 DAS
Grafted plants									
0	0.25±0.14^{a}	3.25±0.38^{bc}	9.75±0.95^{b}	14.58±0.94^{b}	17.58±1.34^{b}	20.58±1.45^{b}	17.17±1.92^{b}	16.42±1.33^{b}	19.50±1.00^{b}
100	0.25±0.14^{a}	2.17±0.30^{cd}	5.92±0.33^{d}	8.08±0.85^{c}	13.08±1.24^{c}	9.92±0.94^{d}	6.92±1.17^{cd}	10.33±1.09^{c}	14.33±1.09^{cd}
200	0.08±0.08^{a}	0.75±0.38^{e}	1.83±0.08^{f}	3.75±0.76^{d}	4.42±0.30^{e}	6.25±1.01^{ef}	4.08±0.79^{d}	5.25±0.52^{d}	6.92±0.67^{e}
300	0.42±0.22^{a}	0.50±0.29^{e}	2.50±0.25^{f}	3.67±0.55^{d}	4.50±0.63^{e}	4.75±0.76^{f}	4.67±0.65^{d}	4.33±0.44^{d}	6.17±0.30^{e}
Non-grafted plants									
0	0.67±0.22^{a}	7.25±0.66^{a}	14.92±0.67^{a}	22.50±0.95^{a}	28.83±1.53^{a}	31.33±1.04^{a}	35.08±1.47^{a}	29.50±1.09^{a}	30.25±1.13^{a}
100	0.25±0.25^{a}	4.00±0.52^{b}	7.67±0.36^{c}	12.33±1.04^{b}	11.75±0.95^{c}	13.33±0.98^{c}	9.17±1.16^{c}	14.50±0.95^{b}	16.08±1.01^{c}
200	0.08±0.08^{a}	1.42±0.22^{de}	4.17±0.22^{e}	6.42±0.73^{c}	7.58±0.55^{d}	8.42±0.58^{de}	5.33±0.88^{d}	8.75±0.66^{c}	12.08±0.65^{d}
300	0.33±0.08^{a}	2.00±0.14^{d}	5.42±0.51^{de}	7.17±0.55^{c}	8.17±0.60^{d}	5.92±0.82^{ef}	5.17±0.93^{d}	8.00±0.58^{c}	13.00±0.72^{d}
Mean grafting									
Grafted	0.25±0.08^{a}	1.67±0.37^{b}	5.00±0.98^{b}	7.52±1.38^{b}	9.90±1.76^{b}	10.38±1.92^{b}	8.21±1.68^{b}	9.08±1.50^{b}	11.73±1.70^{b}
Non-grafted	0.33±0.10^{a}	3.67±0.71^{a}	8.04±1.27^{a}	12.10±1.97^{a}	14.08±2.65^{a}	14.75±3.02^{a}	13.69±3.79^{a}	15.19±2.63^{a}	17.85±2.24^{a}
Mean ZnO-NPs									
0	0.46±0.15^{a}	5.25±0.96^{a}	12.33±1.27^{a}	18.54±1.87^{a}	23.21±2.68^{a}	25.96±2.53^{a}	26.13±4.15^{a}	22.96±3.03^{a}	24.88±2.50^{a}
100	0.25±0.13^{a}	3.08±0.49^{b}	6.79±0.45^{b}	10.21±1.12^{b}	12.42±0.76^{b}	11.63±0.98^{b}	8.04±0.89^{b}	12.42±1.13^{b}	15.21±0.77^{b}
200	0.08±0.05^{a}	1.08±0.25^{c}	3.00±0.53^{c}	5.08±0.76^{c}	6.00±0.76^{c}	7.33±0.71^{c}	4.71±0.60^{c}	7.00±0.87^{c}	9.50±1.23^{c}
300	0.38±0.11^{a}	1.25±0.37^{c}	3.96±0.70^{c}	5.42±0.86^{c}	6.33±0.91^{c}	5.33±0.57^{c}	4.92±0.52^{c}	6.17±0.88^{c}	9.58±1.57^{c}
F value									
Grafting(G)	0.50ns	51.49***	75.59***	63.52***	36.30***	40.05***	42.64***	94.76***	100.51***
ZnO-NPs (Zn)	1.92ns	48.75***	143.85***	118.97***	133.80***	181.32***	148.72***	151.92***	140.18***
G*Zn	0.92ns	6.50**	4.56*	4.05*	14.09***	9.89**	24.60***	13.81***	9.37**

***DAS= Days After Spraying. Means (± SE) in each column followed by different letters are significantly different (Duncan's Test, p ≤ 0.05). ns: not significant; *** significant at p ≤ 0.001, ** significant at p ≤ 0.01, and * significant at p ≤ 0.05.**

Table 2. The influence of foliar application of ZnO-NPs and grafting technique on the egg stage counts of the two-spotted spider mite in cucumber.

ZnO-NPs (mg L^{-1})	10 DAS*	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS	90 DAS
Grafted plants									
0	0.17±0.17^{a}	5.25±0.63^{b}	14.00±0.63^{b}	18.33±0.74^{b}	21.75±1.38^{b}	27.92±1.52^{b}	29.25±1.73^{b}	25.58±1.72^{b}	32.83±1.06^{b}
100	0.17±0.17^{a}	2.75±0.14^{c}	6.08±0.82^{de}	10.83±0.88^{c}	14.58±0.42^{c}	18.33±1.39^{d}	19.50±1.94^{c}	16.08±1.69^{cd}	21.17±0.88^{c}
200	0±0^{a}	0.92±0.46^{d}	3.25±0.38^{f}	4.17±0.51^{d}	7.58±0.88^{de}	8.50±0.63^{f}	10.17±1.48^{d}	11.50±0.88^{e}	13.83±1.16^{e}
300	0.08±0.08^{a}	1.08±0.55^{d}	4.08±0.33^{ef}	4.58±0.96^{d}	6.92±0.71^{e}	9.08±1.23^{f}	14.00±0.76^{d}	11.83±0.36^{e}	12.67±0.94^{e}
Non-grafted plants									
0	0.17±0.08^{a}	10.25±0.66^{a}	21.33±1.17^{a}	27.92±1.66^{a}	33.33±1.88^{a}	40.00±1.75^{a}	50.17±1.36^{a}	46.83±1.20^{a}	44.58±1.47^{a}
100	0±0^{a}	6.17±0.36^{b}	10.08±0.30^{c}	9.92±0.79^{c}	17.58±0.46^{c}	22.67±1.09^{c}	25.58±1.56^{b}	18.33±0.96^{c}	22.92±1.18^{c}
200	0±0^{a}	2.42±0.17^{c}	6.75±0.63^{d}	5.67±0.98^{d}	9.83±0.55^{de}	14.33±0.85^{e}	12.50±2.02^{d}	16.58±1.09^{cd}	17.50±0.80^{d}
300	0.17±0.08^{a}	2.92±0.22^{c}	5.50±0.76^{de}	6.75±0.63^{d}	10.33±1.02^{d}	11.92±0.96^{ef}	12.67±1.48^{d}	13.83±1.54^{de}	17.17±0.71^{d}
Mean grafting									
Grafted	0.10±0.06^{a}	2.50±0.56^{b}	6.85±1.31^{b}	9.48±1.77^{b}	12.71±1.86^{b}	15.96±2.45^{b}	18.23±2.26^{b}	16.25±1.80^{b}	20.13±2.46^{b}
Non-grafted	0.08±0.04^{a}	5.44±0.96^{a}	10.92±1.91^{a}	12.56±2.75^{a}	17.77±2.90^{a}	22.23±3.36^{a}	25.23±4.68^{a}	23.90±4.06^{a}	25.54±3.42^{a}
Mean ZnO-NPs									
0	0.17±0.08^{a}	7.75±1.19^{a}	17.67±1.74^{a}	23.13±2.29^{a}	27.54±2.79^{a}	33.96±2.89^{a}	39.71±4.78^{a}	36.21±4.84^{a}	38.71±2.75^{a}
100	0.08±0.08^{a}	4.46±0.78^{b}	8.08±0.98^{b}	10.38±0.57^{b}	16.08±0.73^{b}	20.50±1.25^{b}	22.54±1.76^{b}	17.21±1.01^{b}	22.04±0.76^{b}
200	0±0^{a}	1.67±0.40^{c}	5.00±0.85^{c}	4.92±0.60^{c}	8.71±0.68^{c}	11.42±1.39^{c}	11.33±1.24^{c}	14.04±1.30^{c}	15.67±1.03^{c}
300	0.13±0.06^{a}	2.00±0.49^{c}	4.79±0.49^{c}	5.67±0.71^{c}	8.63±0.95^{c}	10.50±0.94^{c}	13.33±0.80^{c}	12.83±0.84^{c}	14.92±1.14^{c}
F value									
Grafting(G)	0.09ns	87.58***	70.29***	20.96***	48.56***	52.34***	38.98**	73.80***	53.10***
ZnO-NPs (Zn)	1.06ns	80.23***	155.60***	156.43***	150.61***	157.83***	133.25***	150.38***	221.22***
G*Zn	0.58ns	6.57**	6.40**	11.32***	9.06**	5.50*	18.94***	26.58***	8.67**

***DAS= Days After Spraying. Means (± SE) in each column followed by different letters are significantly different (Duncan's Test, p ≤ 0.05). ns: not significant; *** significant at p ≤ 0.001, ** significant at p ≤ 0.01, and * significant at p ≤ 0.05.**

Table 3. The influence of foliar application of ZnO-NPs and grafting technique on cumulative-mite day (CMD), prevalence, and abundance of its associated predatory mites in cucumber.

ZnO-NPs	Two-spotted spider mite		Predators abundance	
(mg L^{-1})	CMD	Prevalence (%)	*Phytoseiulus persimilis*	*Neoseiulus cucumeris*
Grafted plants				
0	2677.92±77.10^{b}	66.67±3.21^{a}	2.80±0.20^{c}	0.39±0.03^{bc}
100	1625.42±66.87^{d}	55.56±1.60^{a}	7.34±0.41^{ab}	0.51±0.04^{a}
200	828.33±35.45^{f}	41.66±2.78^{a}	1.50±0.16^{d}	0.22±0.02^{d}
300	861.67±33.79^{f}	42.59±3.34^{a}	3.75±0.28^{c}	0.28±0.02^{cd}
Non-grafted plants				
0	4370.83±59.01^{a}	83.33±1.60^{a}	2.99±0.29^{c}	0.31±0.02^{cd}
100	2027.08±80.35^{c}	63.89±4.24^{a}	3.97±0.52^{c}	0.28±0.06^{cd}
200	1250.00±29.24^{e}	51.85±3.34^{a}	6.46±0.14^{b}	0.49±0.05^{ab}
300	1210.83±41.06^{e}	56.48±1.85^{a}	8.19±0.64^{a}	0.25±0.05^{d}
Mean grafting				
Grafted	1498.33±228.01^{b}	51.62±3.32^{b}	3.85±0.67^{b}	0.35±0.03^{a}
Non-grafted	2214.69±388.69^{a}	63.89±3.84^{a}	5.40±0.64^{a}	0.33±0.03^{a}
Mean ZnO-NPs				
0	3524.38±381.03^{a}	75.00±4.06^{a}	2.89±0.16^{c}	0.35±0.02^{a}
100	1826.25±101.25^{b}	59.72±2.75^{b}	5.66±0.81^{a}	0.39±0.06^{a}
200	1039.17±96.50^{c}	46.76±2.99^{c}	3.98±1.11^{b}	0.36±0.06^{a}
300	1036.25±81.62^{c}	49.54±3.54^{c}	5.97±1.04^{a}	0.26±0.02^{b}
F value				
Grafting (G)	324.64***	36.04***	35.28***	0.51ns
ZnO-NPs (Zn)	869.56***	39.09***	30.52***	4.45*
G*Zn	67.18***	0.83ns	55.90***	16.45***

Means (± SE) in each column followed by different letters are significantly different (Duncan's Test, $p \leq 0.05$). ns: not significant; * significant at $p \leq 0.001$, ** significant at $p \leq 0.01$, and * significant at $p \leq 0.05$.CMD = Σ {[0.5 *(A_a + A_{a+1})]*D}, where, A_a: Number of mites on sample "a"; A_{a+1}: Number of mites on next sample date (a+1); D: Number of days between sample "a" and sample "a+1".**

The foliar application of ZnO-NPs markedly enhanced the populations of both *P. persimilis* and *N. cucumeris*. It is also worth to note that the highest density of *P. persimilis* was recorded in plants treated with 300 mg L^{-1}, showing an increase of approximately 106.57% compared to the control group. The plants treated with 100 and 200 mg L^{-1} demonstrated population density increases of approximately 95.85% and 37.72% compared to the control group, respectively. The density of *N. cucumeris* similarly increased, where its highest densities recorded in plants treated with 100 and 200 mg L^{-1}. The density at these concentrations exceeded that of the control group by 11.43% and 2.86%, respectively. At a higher concentration of 300 mg L^{-1}, the density of *N. cucumeris* decreased by approximately 25.71% compared to the control group.

The combined effect of grafting and foliar application of ZnO-NPs significantly influenced the population densities of both predators, where *P. persimilis* exhibited the highest population density on non-grafted plants treated with 300 and 200 mg L^{-1}, which were 118% and 331% higher, respectively, than those on grafted plants treated with the same concentrations. Conversely, the lowest *P. persimilis* density was observed on grafted plants treated with 200 mg L^{-1}, 1.50 ± 0.16 individuals/leaf, compared to 2.99 ± 0.29 and 2.80 ± 0.20 individuals/leaf for the non-grafted and grafted control groups, respectively.

Concerning *N. cucumeris*, the highest density was recorded on grafted plants treated with 100 mg L^{-1}, followed by non-grafted plants treated with 200 mg L^{-1} compared to other treatments. The lowest *N. cucumeris* density was observed on non-grafted and grafted plants treated with the highest ZnO-NP concentration (300 mg L^{-1}).

Accordingly, this study represented the preliminary investigation into the effectiveness of ZnO-NPs and grafting in protecting cucumber plants from TSSM attacks while simultaneously promoting the presence of its natural predators. Earlier studies primarily focused on ZnO-NPs as a control agent –acaricides– against TSSM with the evaluation of its impact on associated predators (Senbill *et al*., 2023; Al-Azzazy *et al*., 2024). Research directly examining the combined effects of ZnO-NPs application and grafting on predatory mites unfortunately is still limited so far. This study presents a novel investigation into how these factors influence the performance of natural predators. The nutritional value of cucumber plants may indirectly influence the nutritional quality of TSSM as prey for predatory mites (West and Nansen, 2014). Recent studies indicate that ZnO-NPs can improve the nutritional composition of crops, potentially affecting pest populations. Consistent with our findings, Uresti-Porras *et al*. (2021) demonstrated that the application of foliar ZnO-NPs and grafting technique together enhanced various nutrient contents and antioxidant activity in bell pepper plants. This rendered the plants less appealing to pests while enhancing their attractiveness to predatory mites. Previous studies indicate that TSSM feeding on cucumber leaves prompts the plants to emit volatile compounds and enhances the production of cucurbitacins (terpenoids), which in turn attract the predatory mite *P. persimilis* (Takabayashi *et al*., 1994; Bouwmeester *et al*., 1999; Agrawal *et al*., 2002; Kappers *et al*., 2011).

Leaf macronutrient and Zn contents

Table (4) illustrates the leaf N, P, K, and Zn content under grafting and foliar ZnO-NPs treatments. The combined effect of grafting and foliar application of ZnO-NPs significantly affected the P and Zn contents but did not significantly influence the N or K contents. The highest P content was observed in the non-grafted plants treated with ZnO-NPs at 300 and 200 mg L^{-1} concentrations, yielding averages of 3.44 ± 0.28 and 2.43 ± 0.20 mg g^{-1} and followed by grafted plants at the same concentrations, respectively. Grafted plants treated with 300 mg L^{-1} had the highest Zn content, which averaging 1.18 ± 0.05 ppm and surpassing other treatments.

These results are in accordance with Li *et al*. (2021) who reported that foliar application of ZnO-NPs at 100 mg L^{-1} significantly increased Zn content in cucumber leaves. They also indicated that ZnO-NPs treatment regulated the uptake and accumulation of micronutrients, thereby positively influencing plant growth and development. Similarly, Jain *et al*. (2013) found that changes in Zn homeostasis also affected the homeostasis of other macro- (P, K) and micro- (Fe, Cu) nutrients.

Table 4. The influence of foliar application of ZnO-NPs and grafting technique on leaves macronutrients and zinc contents of cucumber.

ZnO-NPs (mg L^{-1})	N	P (mg g^{-1})	K	Zn (PPM)
Grafted plants				
0	20.07±0.43^{a}	1.52±0.09^{c}	5.94±0.92^{a}	0.20±0.04^{e}
100	22.12±0.87^{a}	1.56±0.19^{c}	7.37±0.92^{a}	0.32±0.03^{de}
200	24.80±0.83^{a}	2.16±0.27^{bc}	6.11±1.03^{a}	0.72±0.07^{c}
300	25.53±0.68^{a}	2.16±0.19^{bc}	5.61±0.88^{a}	1.18±0.05^{a}
Non-grafted plants				
0	15.97±0.47^{a}	1.45±0.10^{c}	6.06±0.63^{a}	0.18±0.05^{e}
100	18.67±0.93^{a}	1.75±0.30^{bc}	5.56±0.62^{a}	0.39±0.06^{d}
200	22.90±0.67^{a}	2.43±0.20^{b}	6.22±0.56^{a}	0.65±0.03^{c}
300	22.73±0.57^{a}	3.44±0.28^{a}	5.83±0.73^{a}	0.95±0.04^{b}
Mean grafting				
Grafted	23.13±0.72^{a}	1.85±0.13^{b}	6.26±0.45^{a}	0.61±0.12^{a}
Non-grafted	20.07±0.92^{b}	2.27±0.25^{a}	5.92±0.28^{a}	0.54±0.09^{a}
Mean ZnO-NPs				
0	18.02±0.96^{c}	1.49±0.06^{c}	6.00±0.50^{a}	0.19±0.03^{d}
100	20.39±0.96^{b}	1.66±0.16^{c}	6.47±0.64^{a}	0.36±0.03^{c}
200	23.85±0.64^{a}	2.30±0.16^{b}	6.17±0.53^{a}	0.68±0.04^{b}
300	24.13±0.74^{a}	2.80±0.32^{a}	5.72±0.51^{a}	1.07±0.06^{a}
F value				
Grafting (G)	38.00***	7.49*	0.36ns	3.54ns
ZnO-NPs (Zn)	34.73***	15.70***	0.30ns	132.62***
G*Zn	0.89ns	3.76*	0.75ns	3.43*

Means (± SE) in each column followed by different letters are significantly different (Duncan's Test, p ≤ 0.05). ns: not significant; * significant at p ≤ 0.001, ** significant at p ≤ 0.01, and * significant at p ≤ 0.05.**

Leaf biochemical components

This study showed that the combined effect of grafting and foliar application of ZnO-NPs significantly influenced the total phenol, Chl. a+b, and carotenoid contents but did not significantly influence the total carbohydrates or protein content (Table 5). The highest total phenol content (2.35 ± 0.06 mg GAE g^{-1} DW) was recorded in grafted plants treated with 200 mg L^{-1}, followed by those treated with 300 mg L^{-1}. The values surpassed those of non-grafted plants treated with the same concentrations and control groups. The recent results indicated that the combination of cucumber grafting and ZnO-NPs application resulted in a reduced density of TSSM, corresponding with an increase in phenolic contents.

Table 5. The influence of foliar application of ZnO-NPs and grafting technique on leaf biochemical components of cucumber.

ZnO-NPs (mg L^{-1})	Total phenols (mg GAE g^{-1} DW)	Total carbohydrates (mg g^{-1} FW)	Total proteins (mg g^{-1} FW)	Chlorophyll a+b (mg 100 g^{-1} FW)	Carotenoids (mg 100 g^{-1} FW)
Grafted plants					
0	1.81±0.07^{cd}	13.61±0.32^{a}	24.43±0.83^{a}	49.96±2.24^{e}	17.44±0.86^{c}
100	1.92±0.04^{bc}	13.91±0.18^{a}	25.43±0.66^{a}	53.03±1.32^{de}	18.78±0.93^{c}
200	2.35±0.06^{a}	14.51±0.44^{a}	25.03±1.05^{a}	85.76±2.81^{a}	27.31±1.27^{a}
300	2.07±0.08^{b}	14.91±0.38^{a}	24.53±0.71^{a}	69.04±3.38^{b}	22.71±1.12^{b}
Non-grafted plants					
0	1.74±0.03^{d}	11.93±0.49^{a}	22.53±0.44^{a}	47.24±2.67^{e}	18.05±0.81^{c}
100	1.83±0.05^{cd}	13.53±0.38^{a}	25.03±0.77^{a}	48.01±2.64^{e}	17.42±0.94^{c}
200	1.97±0.06^{bc}	13.81±0.34^{a}	24.83±0.77^{a}	60.26±1.42^{cd}	20.48±1.20^{bc}
300	1.98±0.05^{bc}	13.73±0.20^{a}	24.43±0.94^{a}	61.90±2.86^{bc}	20.86±1.22^{bc}
Mean grafting					
Grafted	2.04±0.07^{a}	14.24±0.21^{a}	24.86±0.37^{a}	64.45±4.44^{a}	21.56±1.24^{a}
Non-grafted	1.88±0.04^{b}	13.25±0.28^{b}	24.21±0.44^{a}	54.35±2.29^{b}	19.20±0.64^{b}
Mean ZnO-NPs					
0	1.78±0.04^{c}	12.77±0.46^{b}	23.48±0.60^{a}	48.60±1.67^{c}	17.75±0.55^{b}
100	1.88±0.03^{c}	13.72±0.21^{a}	25.23±0.46^{a}	50.52±1.73^{c}	18.10±0.66^{b}
200	2.16±0.09^{a}	14.16±0.29^{a}	24.93±0.58^{a}	73.01±5.87^{a}	23.89±1.72^{a}
300	2.03±0.05^{b}	14.32±0.33^{a}	24.48±0.53^{a}	65.47±2.54^{b}	21.79±0.85^{a}
F value					
Grafting (G)	16.96**	15.35**	1.36ns	32.36***	9.94*
ZnO-NPs (Zn)	19.17***	7.67**	1.88ns	44.20***	15.77***
G*Zn	3.82*	1.28ns	0.57ns	8.63**	4.48*

Means (± SE) in each column followed by different letters are significantly different (Duncan's Test, p ≤ 0.05). ns: not significant; * significant at p ≤ 0.001, ** significant at p ≤ 0.01, and * significant at p ≤ 0.05.**

This suppression may be attributed to the characteristics of nanoparticles in inducing alterations in the plant's defense mechanisms, including increased production of secondary metabolites like phenols (Li *et al*., 2021). Elevated phenol concentrations in plant leaves have demonstrated the ability to suppress TSSM development and reduce their daily egg-laying rates (Fürstenberg-Hägg *et al*., 2013; Yousuf *et al*., 2024). Phenolics can disrupt the digestive enzymes of mites by binding to and inactivating them, resulting in delayed growth and molting process, which ultimately contributes to reduced mite populations. Higher levels of phenolic compounds have been shown to enhance the defensive capabilities of plants by serving as feeding deterrents, thereby reducing the palatability of plant tissue to spider mites (Mammadova *et al*., 2023). Furthermore, they enhance the synthesis of supplementary defensive

metabolites, resulting in a synergistic effect that bolsters plant resistance (Kumar *et al*., 2020). Research suggests that phenolic compounds may attract natural mite predators, thereby indirectly decreasing spider mite populations (Chen *et al*., 2018).

The present findings clearly showed that the highest Chl. a+b content was recorded at 200 mg L^{-1} (85.76 ± 2.81 mg 100 g^{-1} FW), followed by 300 mg L^{-1} (69.04 ± 3.38 mg 100 g^{-1} FW), with grafted plants outperforming non-grafted plants at the same concentrations. A similar pattern was observed for carotenoid contents. Therefore, the results are consistent with the findings of Ghani *et al*. (2022) who revealed that foliar application of ZnO-NPs at 100 mg L^{-1} leads to the highest photosynthetic activity in cucumber. Also, these results agree with those obtained by De La Rosa *et al*. (2013) and Gupta *et al*. (2022).

Plant growth and yield components

The present study showed that the combined effect of grafting and foliar application of ZnO-NPs significantly increased plant length, LDM%, fruit number, fruit weight, and unmarketable fruit weight percent (Table 6). The greatest increase in plant length observed in grafted plants at 300 mg L^{-1} ZnO-NPs compared to other treatments. The shortest plant length noticed in non-grafted plants treated with 100 mg L^{-1} and in both grafted and non-grafted control groups. Similarly, LDM% was highest in grafted plants at all ZnO-NPs concentrations tested, followed by non-grafted plants treated with 300 mg L^{-1}.

Table 6. The influence of foliar application of ZnO-NPs and grafting technique on plant growth and yield components of cucumber.

ZnO-NPs (mg L^{-1})	Plant growth		Total yield per plant			
	Plant length (m)	Leaves DM (%)	Fruit Number	Fruit wt. (Kg)	Average fruit wt. (g)	Unmarketable fruit wt. (%)
Grafted plants						
0	2.10±0.03^{b}	22.33±0.32^{b}	34.26±0.55^{e}	2.23±0.02^{e}	65.22±1.27^{a}	17.76±0.56^{b}
100	2.20±0.14^{b}	22.25±0.77^{b}	41.60±1.00^{c}	2.85±0.03^{c}	68.48±1.19^{a}	10.10±0.15^{d}
200	2.60±0.04^{a}	25.21±0.79^{a}	44.97±0.89^{b}	3.16±0.03^{b}	70.21±1.46^{a}	8.07±0.70^{ef}
300	2.70±0.06^{a}	23.35±0.29^{ab}	47.62±0.76^{a}	3.43±0.05^{a}	72.01±1.41^{a}	7.12±0.29^{f}
Non-grafted plants						
0	1.75±0.03^{d}	17.45±0.60^{d}	31.36±0.38^{f}	1.83±0.04^{f}	58.46±1.39^{a}	25.38±0.60^{a}
100	1.75±0.03^{d}	18.09±0.87^{cd}	33.33±0.33^{ef}	2.25±0.03^{e}	67.58±0.63^{a}	13.82±0.63^{c}
200	1.80±0.04^{cd}	19.55±0.74^{c}	38.50±0.75^{d}	2.60±0.01^{d}	67.60±1.61^{a}	10.33±0.92^{d}
300	2.00±0.14^{bc}	21.97±0.43^{b}	42.18±0.92^{c}	2.86±0.03^{c}	67.84±0.87^{a}	9.67±0.46^{de}
Mean grafting						
Grafted	2.40±0.08^{a}	23.29±0.44^{a}	42.12±1.55^{a}	2.92±0.13^{a}	68.98±0.95^{a}	10.76±1.28^{b}
Non-grafted	1.82±0.04^{b}	19.26±0.60^{b}	36.35±1.31^{b}	2.39±0.12^{b}	65.37±1.31^{b}	14.80±1.92^{a}
Mean ZnO-NPs						
0	1.92±0.08^{b}	19.89±1.13^{b}	32.81±0.71^{d}	2.03±0.09^{d}	61.84±1.73^{b}	21.57±1.74^{a}
100	1.97±0.12^{b}	20.17±1.07^{b}	37.47±1.91^{c}	2.55±0.13^{c}	68.03±0.64^{a}	11.96±0.88^{b}
200	2.20±0.18^{a}	22.38±1.36^{a}	41.74±1.54^{b}	2.88±0.13^{b}	68.90±1.13^{a}	9.20±0.72^{c}
300	2.35±0.17^{a}	22.66±0.39^{a}	44.90±1.33^{a}	3.14±0.13^{a}	69.93±1.19^{a}	8.39±0.62^{c}
F value						
Grafting (G)	110.98***	79.29***	122.87***	553.12***	16.26**	96.00***
ZnO-NPs (Zn)	13.45***	10.22**	101.80***	451.55***	16.53***	216.11***
G*Zn	3.69*	4.26*	4.64*	3.78*	1.93ns	9.01**

Means (± SE) in each column followed by different letters are significantly different (Duncan's Test, $p \leq 0.05$). ns: not significant; * significant at $p \leq 0.001$, ** significant at $p \leq 0.01$, and * significant at $p \leq 0.05$.**

As regards yield components, the highest fruit number (47.62 ± 0.76 fruits/plant) was observed in grafted plants treated with 300 mg L^{-1}, followed by those treated with 200 mg L^{-1} (44.97 ± 0.89 fruits/plant) compared to non-grafted plants treated with the same concentrations of ZnO-NPs. Similarly, the grafted plants treated with 300 mg L^{-1} produced the highest total fruit weight per plant (3.43 ± 0.05 Kg/plant) compared to non-grafted plants (2.86 ± 0.03 Kg/plant) at the same concentration. The results also showed that the grafted plants had the lowest percentage of unmarketable yield when treated with 300 and 200 mg L^{-1}, averaging 7.12 ± 0.29% and 8.07 ± 0.70%, respectively, followed by the non-grafted plants treated with 300 mg L^{-1}, with an average of 9.67 ± 0.46%. The percentage of unmarketable yield increased by 25.38 ± 0.60% in non-grafted control plants and by 17.76 ± 0.56% in grafted control plants.

The present results align with Gupta *et al*. (2022) who demonstrated that foliar applications of ZnO-NPs at 300 mg L^{-1} during three growth stages of cucumber enhanced plant height, fruit number, and weight. Similarly, Li *et al*. (2021) established that the application of ZnO-NPs as a foliar spray enhanced cucumber plant performance at a concentration of 100 mg L^{-1}, evidenced by increases in both fresh and dry leaf weights. This aligns with De La Rosa *et al*. (2013) who indicated that foliar application of ZnO-NPs improved yield as well as biomass production of cucumber. In addition, Nisar *et al*. (2022) showed that cucumber plant height and yield were increased in plants treated with foliar Zn-NPs at 20 ppm. This concentration was superior to that of 10 and 30 ppm Zn-NPs. Similarly, Hussein and Khalaf (2022) showed that spraying cucumber with Zn-NPs at 150 mg L^{-1} recorded the highest plant length, LDM%, fruit weight, plant yield, and total yield of greenhouse cucumber compared to the control treatment. This denotes that the application of zinc as a nano-fertilizer promotes plant growth. A study of Magdaleno-García *et al*. (2022) showed that the combined application of ZnO-NPs and grafting has been shown to promote various growth parameters in grafted eggplants, including plant height, stem diameter, number of leaves, and dry weight. Similarly, the combination of grafting and selenium NPs enhanced the productivity of grafted cucumber plants (López *et al*., 2021).

CONCLUSION

The synergistic effects of grafting and application of ZnO-NPs proved particularly effective in suppressing the spider mite population and enhancing the presence of natural predators in cucumber. Grafting has altered the plant's

physiological and biochemical properties, making it more receptive to nanoparticle treatment and enhancing its defensive responses against spider mite infestation. This illustrates the multifaceted benefits of grafting in sustainable crop production and protection against detrimental pests such as TSSM.

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التأثيرغير المباشر لجسيمات أكسيد الزنك النانوية وتقنية التطعيم علي أداء عشائر العنكبوت الأحمر ذي البقعتين ونمو نباتات الخيار النامية في الصوب

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الملخص

تحظى جسيمات أكسيد الزنك النانوية (ZnO-NPs) بإهتمام متزايد كمعزز لإنبات البذور ونمو النبات وقوة الشتلات، فضلاً عن دورها في مكافحة آفات المحاصيل. قمنا في الدراسة الحالية بتقييم التأثير غير المباشر لإستخدام أكسيد الزنك النانوي (كسماد تكميلي) وتقنية التطعيم على ملائمة نباتات الخيار للعنكبوت الأحمر ذي البقعتين *Tetranychus urticae* Koch وأعدائه الطبيعية في الصوب. كما تم تقييم التأثير علي بعض الصفات الخضرية، والبيوكيمائية والمحصولية للخيار. تم رش أوراق نباتات الخيار بأربعة تركيزات من أكسيد الزنك النانوي: صفر (الكنترول)، 100، 200، و300 مجم/ لتر علي ثلاث دورات. كانت فعالية أكسيد الزنك النانوي في قمع كثافة البيض والاطوار المتحركة للعنكبوت الأحمر معتمدة على التركيز. أشارت النتائج الي أن النباتات المطعومة المعاملة بتركيزي 200 و300 مجم/ لتر أظهرت أقل عدد من البيض والأطوار للعنكبوت الأحمر مقارنة بالنباتات غير المطعومة. أدي الرش بأكسيد الزنك النانوي الي زيادة كثافة المفترسين الأكاروسيين *Phytoseiulus persimilis* A.-H و(Oudemans) *Neoseiulus cucumeris* . أظهرت النتائج أيضاً أن تأثير التفاعل بين التطعيم وأكسيد الزنك النانوي قد عزز معنوياً طول النبات ونسبة المادة الجافة للأوراق ومحتوى صبغات التمثيل الضوئي والمحصول. تحسنت تركيزات العناصر الغذائية الكبرى والصغري، وبعض الصفات البيوكيميائية في الأوراق بشكل ملحوظ. وبذلك أثبتت النتائج فعالية دمج إستخدام أكسيد الزنك النانوي وتقنية التطعيم في تقليل كثافة العنكبوت الأحمر وتعزيز فعالية أعدائه الطبيعية وتحسين إنتاجية المحصول في نفس الوقت.

الكلمات الدالة: *Tetranychus urticae*، *Phytoseiulus persimilis* ، *Neoseiulus cucumeris*، نانو أكسيد الزنك، التطعيم، العناصر الغذائية الكبري، فينولات