Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Development of insecticidal nano-composite solution to control termite losses in peach

Sana Zarish
PMAS-Arid Agriculture University
Naveed Iqbal Raja
drnaveedraja@uaar.edu.pk
PMAS-Arid Agriculture University
Fozia Abbasi
PMAS-Arid Agriculture University

Research Article

Keywords: Green synthesized Ag/ZnO nanoparticles, Termites, Nanotechnology, Peach tree

Posted Date: October 23rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3409571/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Development of insecticidal nano-composite solution to control termite losses in peach

[1]Sana Zarish, [1]Naveed Iqbal Raja*, [1]Fozia Abbasi.

Sanayaqoob116@gmail.com drnaveedraja@uaar.edu.pk abasifozia@gmail.com

[1]*Department of Botany, PMAS-Arid Agriculture University, Rawalpindi 46300, Pakistan*
* Corresponding author address: drnaveedraja@uaar.edu.pk

Abstract

Peach *(Prunus persica L.)* be a part of family Rosacea, although Aaru is its local name. Pakistan ranked 25th in peach production. In Pakistan, peach is a prime stone fruit after plum. It is summer fruit but can be used in winter season after drying its fruit. It also contains medicinal properties because it carries various phenolic compounds in fruit. Termites are one of the most important insect that damage the peach tree and due to their attack tree should be hollowed, leaves become wilt and ultimately becomes in the form of plant death. Termites attack on plants can be control by a unique innovative technique which is nanotechnology. Nanotechnology is the 21st century science executes output of various nanoparticles possessing range amid 1 to 100 nm. Among these, Ag/ZnO nanoparticles are very important due to raised surface area to volume ratio and catalytic efficiency. Bimetallic nanoparticles may increase water uptake, nutrient use efficiency, hence may improve plant growth and their yield by changing physio-morphic and biochemical attributes. Green bimetallic Ag/ZnO nanoparticles will be synthesized from salts reduced by *Moringaoleifera* Lam. Ag/ZnO nanoparticles will be characterized by UV-Visible Spectroscopy, SEM, FTIR and EDX. Nanocomposite Ag/ZnO with various concentrations i.e 100ppm, 200ppm, 300ppm, 400ppm, 500ppmand have two control (T+ no treatment and T- 500ppm Ag/ZnO salt). Effect will be analyzed for physiological (membrane stability index, chlorophyll content, relative water content), biochemical (SOD, POD, catalase, proline content,

protein content, sugar content). All the obtained data will be analyzed through ANOVA by using SPSS 16.1 program.

Keywords: Green synthesized Ag/ZnO nanoparticles: Termites: Nanotechnology: Peach tree

1. Introduction

Pakistan is an agriculture nation. Agricultural region had conventionally maintained satisfying production to secure the food security for the development of population. This sector comprises the Gross Domestic Product (GDP) of about 19.5% [1]. Fruits contain beneficial sources of vitamins, antioxidants, minerals and consist of low amount of fat. Pakistan possesses an ample heritage of fruit gardens, chiefly in the harvesting period. It kept long self-life because it ca be used as a dry fruit in winter season. Fruits of Pakistan are famous and present all over the world and also contain a prolonged list. Peaches apples, citrus fruit etc are most admired inside and outside the Pakistan. Applications of peach cultivation (*Prunuspersica L.*) began from old days. Peach gives yearly production around 21.08 million tons from cultivated region of 14.9 million hectares. Peaches possess third position in the fruit species after the apple and pears. Recently peach considered fundamental part of horticultural produces. Peaches contain huge amount of nutrients and well known as a summer fruit [2].

Peach those cultivated in Pakistan, is a temperate fruit that contain great economic importance [3]. In Pakistan peach kept second rank in stone fruits and well known as a queen of the fruits. Cultivation of peach in province Punjab is reported on an area of 36 hectares, Khyber Pakhtunkhwa 9013 hectares and in Balochistan 5301 hectares that give productive rate is about 222, 56776 and 15538 tonnes correspondingly in Punjab, KPK and Balochistan [4]. Termites are also known as white ants and considered as insect-pests of fruit crops that present in nursery, newly grown fruit farms and full grown trees in Punjab. In Punjab, *Odontotermesobesus* and

Microtermes spp. are abundantly observed in nurseries and fully grown fruit farms and suppose as common termite. When termite damage the plant, it eventually dried. In Punjab April-June and September-October is considered as peak activity of termite's productivity rate [5]. Termites are present all over the world but absent in Antarctica. Termites cause loss in productivity throughout the world which is about 5.1 to 9.3% from the revolution era of pre-green to post-green. About 200,000 white ants eat approximately 5.4 kg of cellulose yearly because their food dependence occur on cellulose [6]. In Peshawar Valley, stone fruits like peach and plum affected with peach flatheaded borer, *S. dadkhani (Oben.)* which is prime insect pest [7]. Nanotechnology has a magnitude of 1to100nm which roughly control the sizes of matter. If any particle contains dimension of this range then it supposed as a nanoparticle [8]. Presently, nanotechnology is comprehensively used in agriculture and horticulture. It plays crucial function in increasing vegetative, reproductive growth, speed up yield rate, fruit quality and their self-life. It reduces the fruit waste. Nanoparticles are natural or engineered, and can be illustrated whose dependence on their core material. Over the last few years' nanotechnology or nanoscience possessed essential constituents which are nanostructured material and nano particles (NPs). This happens due to their physiochemical properties including size-dependent optical, catalytic properties and potential applications [9].

Traditional insect pest management practices cannot give effective methods for controlling termites or insects, so intellectual approach used which is nanoparticles that provide protection to plants from insect. Moreover, it also used for the synthesis of new compounds against pest. Nanoparticles possess magic bullets. When tissues of plants targeted by magic bullets which filled with herbicides, fungicides, nutrients, then results easily achievable [10]. "The Nanotechnology Consumers Products Inventory" recognizes zinc oxide (ZnO) nanoparticle

as fifth abundantly utilized substance in respect of their usage in consumer products. ZnO nanoparticles are generally utilized in anti-microbial agents, personal care products, photovoltaicsand paints. Specifically, silver nanoparticles possesses immense admiration amongst metallic nanoparticlesdue to their antimicrobial properties manifesting broad spectrum among various areas. The propitious outcomes and expected advantages of silver nanoparticles in agriculture revealed an advance approach of examination in various fields such as forestry and agroforestry, especially for objectives of pest management. Ag/ZnO nanoparticles have ability to take part in the roots of crops through apoplastic pathway and then transferred to shoots by means of vascular system.

Consequently, their uptake relies upon plant anatomy and composition, size, and shape of nanoparticles. Ag/ZnO nanoparticles possess productive impact on plant propagation, maturation or growing stages including leaf area, fertilizer uptake, water absorption, chlorophyll content, phenolic content, nutritious usage efficacy and crop yield. Ag nanoparticles give more attention because it possesses antimicrobial properties and provides satisfactory outcomes in agricultural fields. Ag/ZnO nanoparticles became popular among other nanoparticles due to possession of different properties having more chemical stability, low cost and vast surface area [11-13]

2. Material and methods

Ag/ZnO nanoparticles synthesized according to the Sorbium et al. (2017). For the purpose of formation of Ag/ZnO NPs, take 0.1 M ($ZnSO_4.7H_2O$) (50 ml) and 0.01M $AgNO_3$ (50 ml) were added with plant extract in an Erlenmeyer flask. Solution gives light brown color when it heated for at least four hours at 80°C. Solution would be removed after 4 hour from heat and kept overnight for cooling at room temperature. Next day, at 4000 rpm mixture was used for centrifugation for 20 min. After the removal of supernatant phase, nanoparticles were cleanse with water is about 15 mL for the purpose of removing impurities

or dust at least 3 times. Product will be obtained in the powder form after drying the precipitates for 6 hours by placed them on oven at 80 °C. At 500 °C, powder was used for calcination for at least 4 hours and eventually gray colored product will be obtained. Figure1 show the preparation of nanoparticles. Peach tree planted in chakwal and various concentration of nanocomposite silver zinc oxide was applied on peach plants. There are five treatments and has two control treatment i.e positive and negative control and table 1 explained the experimental layout. Morphological, biochemical and physiological attributes were obtained in PMAS-UAAR research lab of botany department.



Figure 1. Show nanoparticles preparation method.

Treatment	Concentrations of Ag/ZnO NPs

T0 (-VE)	Control
T0 (+VE)	500 ppm\l Ag\ZnO salt
T1	100 ppm\l Ag/ZnO NPs
T2	200 ppm\l Ag/ZnO NPs
T3	300 ppm\l Ag/ZnO NPs
T4	400 ppm\l Ag/ZnO NPs
T5	500 ppm\l Ag/ZnO NPs

Table 1. Show experimental layout.

2.1 Techniques of characterization

Various techniques used for the characterization of green synthesized silver zinc oxide nanoparticles. With the help of sonication, Ag/ZnO nanoparticles were dipped precisely in distill water. Synthesis of Ag/ZnO nanoparticles were observed by noticing UV-visible spectra using HALO DB-20. With the help of SIGMA model, morphology of the green synthesized silver zinc oxide nanoparticles were seen by SEM from University of Peshawar. For SEM, the samples would be synthesized by simply placing minor amount of sample on carbon coated copper gird. Then with the aid of dry air and scanning electron microscopy micrographs, films were obtained on various amplifications by scanning electron microscope (SEM). EDX detector utilized for the elementary analysis of the bimetallic silver zinc oxide alloy nanoparticles by dropping the prepared nanoparticles on carbon film.

2.2 Termite rearing

Termites collected from field. After collecting termites, take a plastic tray or colonial flash that filled with moisture animal manure. Then putted small pieces of dry wood in the tray or flask as well as insects. Cover it with a paper that has small pores or any other source like perforated lid. Keep them at room temperature in a lab. Then within a week, insects would be

reared. In an appropriate amount of insects should leave on shoots and also apply on roots because they eat wood and affect the plant developmental stage. For the purpose of insect control and yield rate enhancement, green synthesized Ag/ZnO nanoparticles applied on the peach tree.

2.3 Morphological Parameter

Leaf area can be estimated by removing leaf from every treatment of plant. With the help of leaf area meter, leaf areas of having each specimen were estimated. Randomly take three peach leaves for the observation of plant fresh weight from each treatment. Fresh weight was calculated individually. For the plant dry weight, take three leaves of each treatment and washed them with tap water. After this, plants were putted in oven at room temperature to record plant dry weight of each treatment.

2.4 Physiological Parameters

Measurement of relative water content will be measured from Wheatherley method. For this purpose, a leaf of 0.2g was taken from each treatment. Measure the fresh weight of leaf and then leaf was placed in petri plate for 24 hours that filled with water. After this turgid weight of leaves would be noted. Then these leaves were placed in oven 70^{o}C for one week. After that, dry weights were calculated after one week.

$$\text{Relative water content} = \frac{(\text{Fresh weight} - \text{Dry weight}) \times 100}{(\text{Saturated weight} - \text{Dry weight})}$$

Membrane stability index will be measured from the method of Sairam [14]. Leaf was taken from each treatment. Leaves will be cutted into (100 mg) little pieces and distilled water used for rinsing at least two times. At 40^{o}C, these small discs were add up into the test tubes which would be set down on bath water for 30 minutes. After that electric conductivity (C_1) was recorded by EC meter. In water bath these test tubes again placed at 100^{o}C for 10 minutes. C_2 (electric conductivity) will be measured after 10 minutes. Membrane stability index measured with the help of following formulation.

$$\text{Membrane stability index} = [1 - C_1 / C_2] \times 100$$

For the evaluation of leaf chlorophyll content, spectrophotometer would be used via applying the method of Bruinsma [15]. Leaf sample (0.2 g) will be grinded in 10 ml of acetone. Filtrate was attained in one more arrangement of test tubes after the completion of crushing. Then level of absorbance will be recorded at wavelength of 645, 652 and 663 nm. Leaf chlorophyll content will be measured by using equations.

Chlorophyll a contents = $12.7(A_{663}) - 2.7(A_{645})$

Chlorophyll b contents = $22.9(A_{645}) - 4.7(A_{663})$

Total chlorophyll contents = $(A_{652} \times 1000/34.5)$

2.5 Biochemical Parameters

Proline contents will be measured comprehensively from the method of Bates et al. [16]through use of spectrophotometer. Grind the healthy leaf samples (0.3 g) in 4ml sulfosalicylic acid (3.0%). In another set of test tubes take 2 ml of filtrate and acquiescing would react to glacial acetic acid and ninhydrin reagent both contain about 2ml. Then it boiled in water bath and after occurrence of color, if tubes are placed on ice, reaction will be ceased. After this 4 ml of toluene were added and solution will be merged properly until upper colored layered is raised. This layer was segregated from solution mixture in another set of test tubes and absorbance of this were measured at 520 nm wavelength. By using successive method proline contents were calculated.

$$\text{Total proline } (\mu g/ml) = \frac{\text{(Sample absorbance} \times \text{Dilution factor} \times \text{K value)}}{\text{Fresh weight of plant tissue}}$$

Phenol sulphuric acid method was use for the estimation of soluble sugar contents (Dubois et al.,[17]. Take leaf which is about is 0.5 g and merged with 80% ethanol whose containing 10ml. After that use water bath for heat for at least 1 hour at 80^{o}C. About 0.5 ml sample extract will be placed in tubes and 18% phenol were add up and at room temperature mixture provided for incubation. After passing 1 hour, 2.6 ml sulphuric acid was added. Then mixture should be shacked efficiently. At the end absorbance of each sample would be recorded

at 490 nm wavelength by using spectrophotometer. With the help of under given formula sugar contents were measured

$$\text{Sugar } (\mu g/ml) = \frac{\text{Sample absorbance} \times \text{Dilution factor} \times \text{K value}}{\text{Weight of fresh plant tissue}}$$

Protein content was measured by taking 0.5g of fresh leaves and then these leaves will be grinded in phosphate buffer. Then extract would be taken little more than 0.5 ml, bio red dye is of 3ml of and 0.5 ml of distilled water were added. The solution will be shacked properly and then absorbance of sample will be observed at 595nm (Lowry et al., [18].

Sairam and Srivastava [19] method was used for measurement of superoxide dismutase contents. In test tube take 10ml of phosphate buffer whose pH 7. Weigh the fresh sample which contains 0.5g and then for the purpose of homogenization it crushed in phosphate buffer. Centrifuge the tubes in centrifuge machine at 15000 rpm for 15 minutes at 4C^{o}.Take 0.1ml sample extract and add up 0.1ml riboflavin stock and 3ml superoxide dismutase buffer. Continuously reaction mixture was shaked and then under florescent lamp place solution for 8 min at 25C^{o}. mixture solution that present in test tubes possesses yellow color, then moved to either dark brown or uniformly black. Synthesized another arrangement of same samples but at that time these are not placed in lights. In dark condition these tubes should be placed. Dark and light sets contain wavelengths that were observed at 560 nm on spectrophotometer.

For the determination of ascorbate peroxidase contents method of Nakano and Asada [20]was used. Take 1g of leaf tissue and then weigh them. Then grind in 2 ml of 50% ethanol individually in a pre-chilled mortar and pestle and the extracts were centrifuged in centrifuge machine at 4ºC for 10 minutes at 10,000 rpm. The obtained supernatants will be able to use within four hours. Supernatants will be treated with 2ml of a solution which contain 1% guaiacol, 40mM H_2O_2 and 100mM phosphate buffer (pH 7) consecutively. The colourful product obtained from enzyme by using H_2O_2 and guaiacol as substrates. The absorbance of product was recorded at 470nm and peroxidase activity will be manifested in form of enzyme activity units (U).

Catalase activity was determined by using a method that explained by Pereira [21]

through analyzing reduce in H_2O_2 contents at 240 nm wavelength. For evaluation of catalase activity frozen sample were taken in appropriate amount that homogenized in 60µL medium comprised of 50mM phosphate buffer and 1 mM DTT. The sample centrifuged for 5 min at 12000 rpm and the supernatant was usable for catalase activity. For essay of enzyme activity, the oxidation rate would be easily get from absorbance at 240nm. The solution mixture comprises of about100µL extract of enzyme and 2ml of distilled water. Catalase activity will be observed by following reduction in absorbance for 3 min at 240nm using spectrophotometer.

$$\text{Catalase (units/ mg protein)} = \frac{\text{A240nm / min} \times 1000}{43.6 \times \text{mg protein / ml reaction mixture}}$$

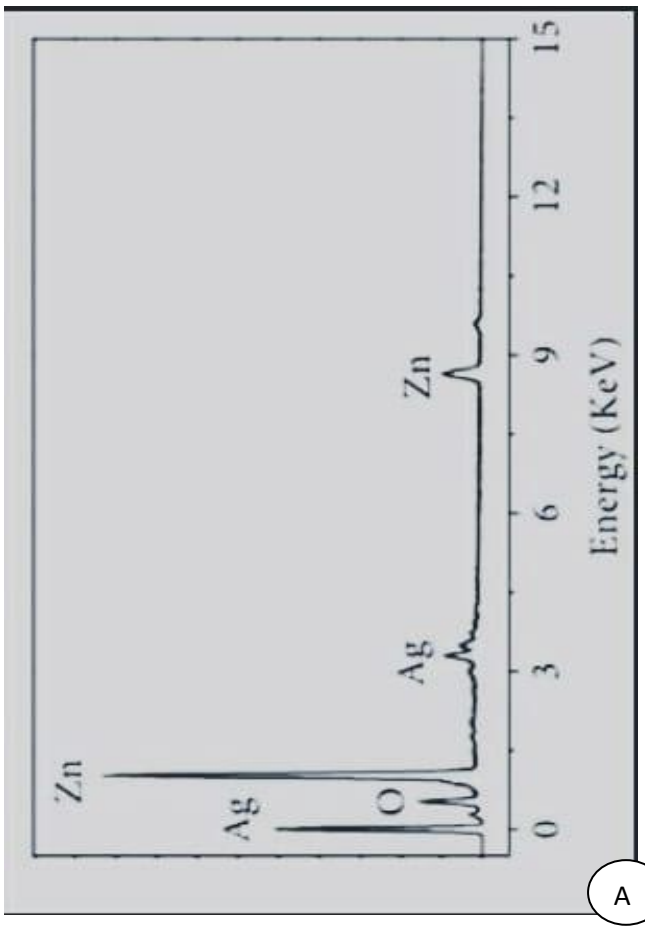
2.6 Statistical Analysis

Each experiment will be repeated twice, and each treatment were comprised of three replicates. In factorial experimental arrangement of treatments all experiments were laid out. Mean values of different treatments will be apted to ANOVA analytical variance. By evaluating the significance, SPSS was used at $p < 0.05$ through LSD.

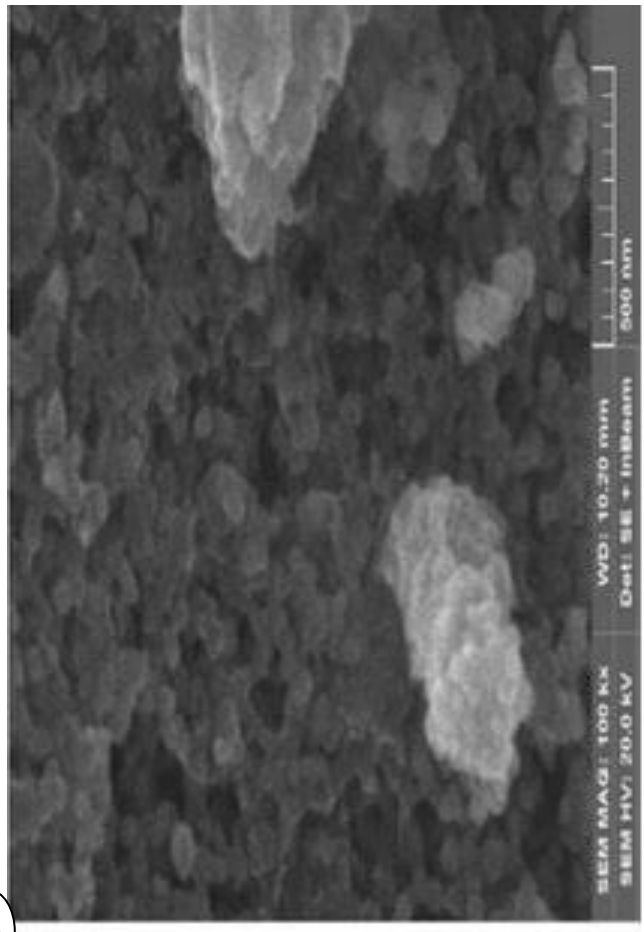
3. Results

3.1 characterization of green synthesized silver zinc oxide nanoparticles

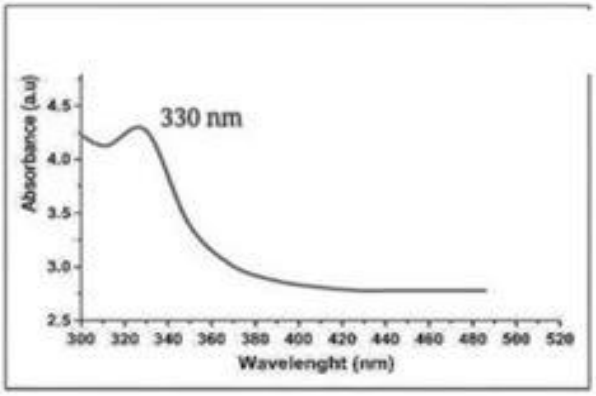
EDX analysis should be used for the purpose of knowing the presence of Ag/ZnO nanoparticles. Highest peak of silver zinc oxide nanoparticle shown in figure 2A. Scanning electron microscopy used for the purpose of structural analysis of Ag/ZnO nanoparticles. It gives spherical shape and has 44 to 62 size range that shown in figure 2B. Synthesis of Ag/ZnO and ZnO nanoparticles were characterized by UV- visible spectrum from the wavelength 366–379 nm and 330 nm on HALO DB-20 spectrophotometer, which represented in figure 2C.

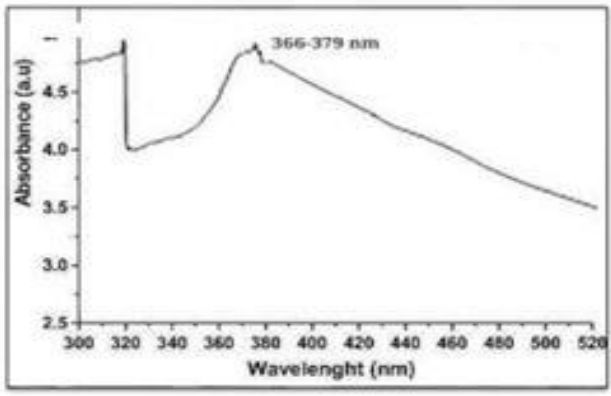

Zn
Ag
O
Ag
Zn
0
3
6
9
12
15
Energy (KeV)

A


SEM MAG: 100 kX
SEM HV: 20.0 kV
WD: 10.20 mm
Det: SE + InBeam
500 nm

B


330 nm
Absorbance (a.u)
4.5
4.0
3.5
3.0
2.5
300 320 340 360 380 400 420 440 460 480 500 520
Wavelenght (nm)


366-379 nm
Absorbance (a.u)
4.5
4.0
3.5
3.0
2.5
300 320 340 360 380 400 420 440 460 480 500 520
Wavelenght (nm)

C



Figure 2. (**A**) Represent the EDX spectrum of Ag/ZnO NPs; (**B**) Show SEM micrograph of Ag/ZnO NPs; (**C**) Show UV- spectrum of Ag/ZnO; (**D**) Show termites collection.

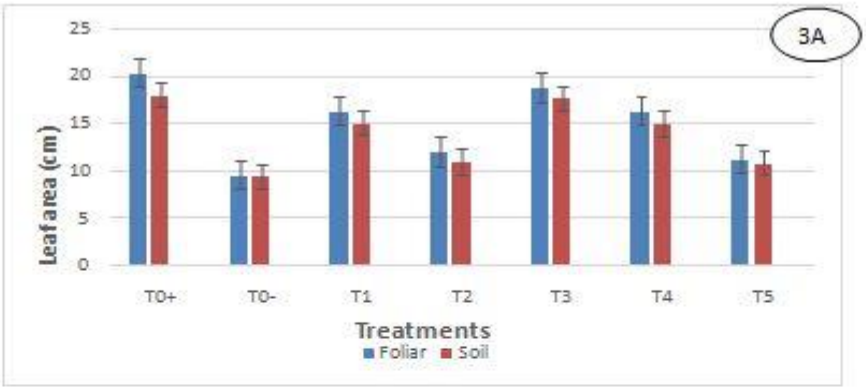
3.2 Role of silver zinc oxide nanopcomposite on morphological attributes

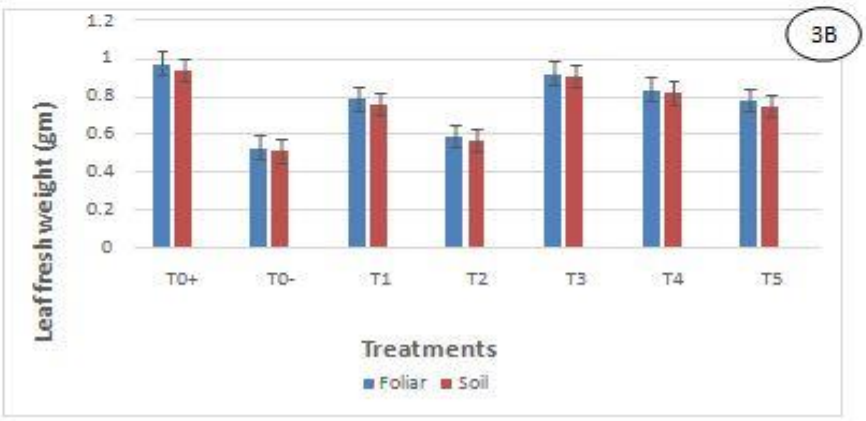
Phenomenal increase in leaf area was observed by the application of Ag/ZnO NPs at 300ppm that plant shows 18.8cm value increases as compared to control in foliar application in figure 3A. The decrease result shown in control plant that was treated with Ag/Zn salt by 9.61cm value. In case of soil application, 300ppm give significant results that plant shows 17.7cm value. While decrease results shown on control plants by 9.45 cm. The overall increased result produced by 1.1 cm value in foliar as compared to soil.

Plants which treated with silver zinc oxide salt cause 0.53gm value in leaf fresh weight as compared to control in foliar while treatment of silver zinc oxide nanoparticles 300ppm show 0.92gm increase in fresh weight (Figure 3B). Remarkable results were obtained in plant with 300ppm as compared to control. In soil application, silver zinc oxide insecticidal nano-composite solution treated on plants, T3 plants show best results (0.91gm) to control termite losses in peach. T0- shows only decreased results in leaf fresh weight.

Silver zinc salt reduces plant dry weight (0.16gm). Best results were observed in 300ppm of insecticidal silver zinc oxide nanoparticles treatment (0.35gm) in foliar whereas in soil

decreased value (0.14gm) recorded in T0- and increased concentration was recorded in 300ppm plants (0.32gm). While greater concentration of nanoparticles show decrease plant dry weight and shown in figure 3c. Fresh and dry weight of plant were only affected with salt show decrease result as compared to control.





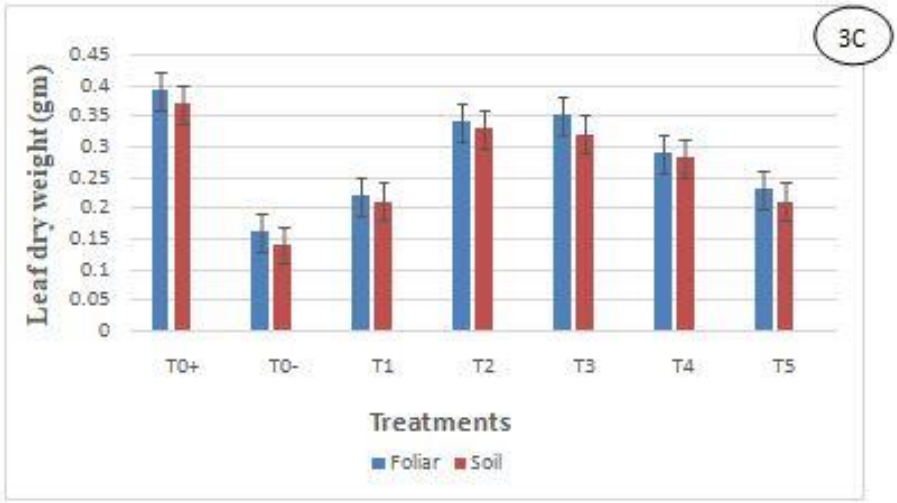


Figure 3. (A) Effect of Nanocomposite Ag/ZnO on peach tree leaf area; **(B)** effect of Ag/ZnO on leaf fresh weight of peach tree; (**C**) effect of Ag/ZnO on leaf dry weight of peach tree by controlling termites.

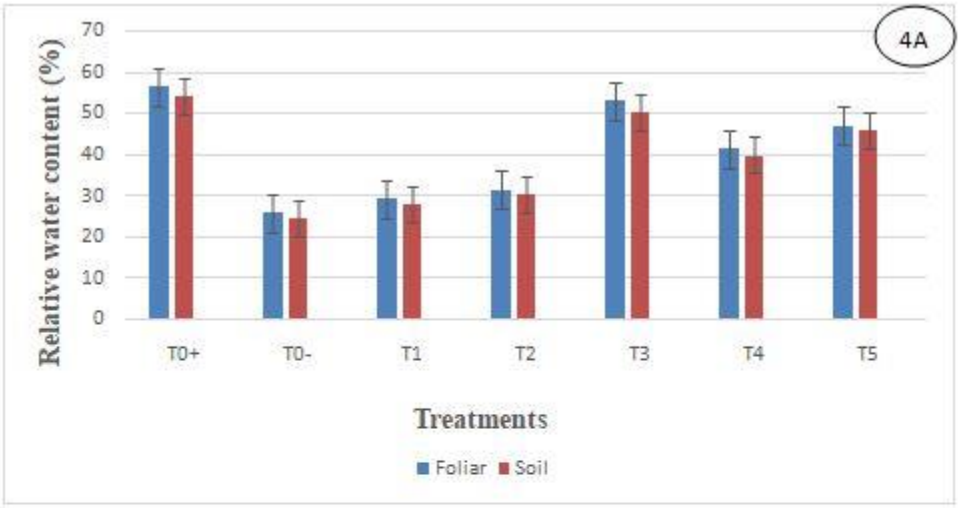
3.3 Effect of Ag/ZnO on Physiological Parameters

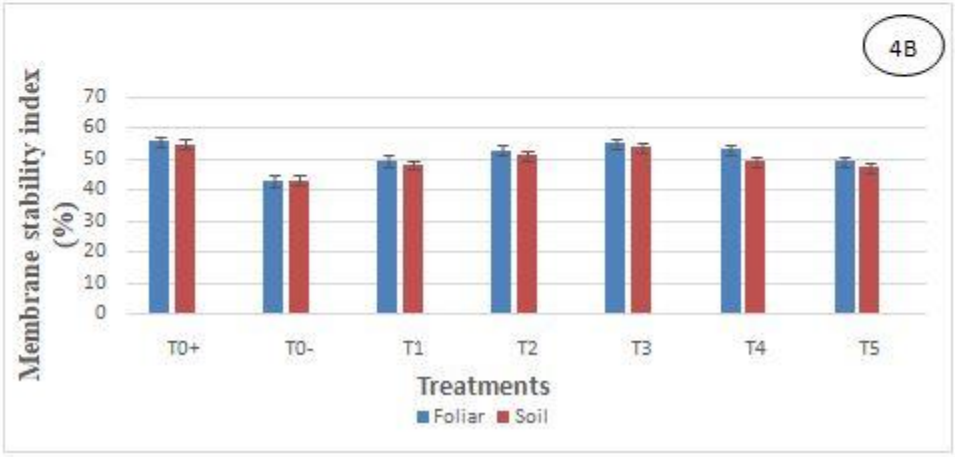
Plant treated with silver zinc salt decreases relative water content (25.9%) in foliar and (24.7%) in soil application (Figure 4A). Higher concentration such as 300ppm give best results of increased relative water content (52.9%) value in foliar and (50.1%) value in soil application. Higher concentration gave higher relative water content than lower concentration. Membrane stability index decreases in plants which treated with silver zinc salt were provided values 42.8% in foliar and 43% in soil application (Figure 4B). Remarkable increase observed in higher concentration of silver zinc oxide nanocomposites to control termite losses in peach. Best result shown in 300ppm treated plants (54.9%) in foliar and (53.6%) in soil application Best reduction in chlorophyll a content occur in plants that treated with salt (18.1 µg/ml) in foliar and (17.4µg/ml) in soil. While increase in chlorophyll a content was observed in 300ppm of silver zinc oxide nanoparticles (26.0 µg/ml) value in foliar and (25.4 µg/ml) in soil. Significant results observed in foliar application.

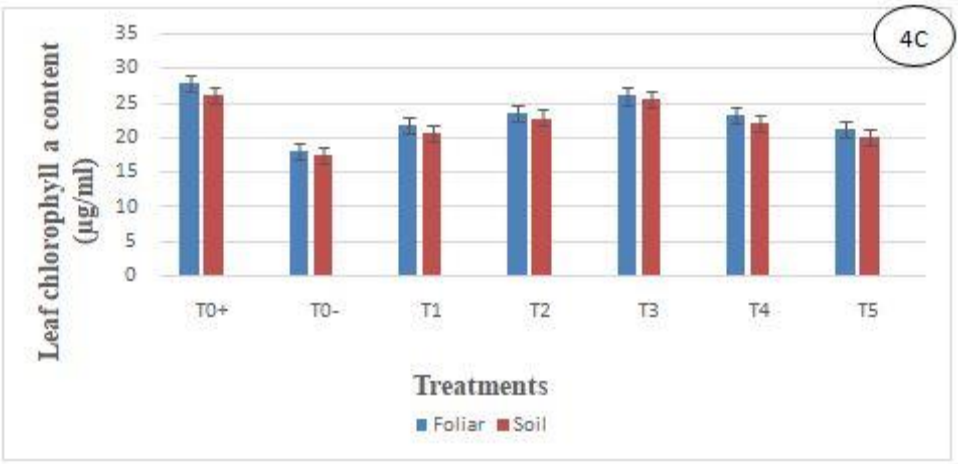
Results regarding application of silver zinc oxide nanoparticles to control termite losses in peach in foliar and soil plants (Figure 4D). Decrease in chlorophyll b content was observed in plant that treated with salt (11.6 µg/ml) in foliar and (10.8µg/ml) in soil application. While pronounced effect were found in 300ppm of silver zinc oxide nanoparticles (25.0 µg/ml) in foliar and (24.1µg/ml) in soil. However, decrease in result in chlorophyll b content in higher as well as lower concentration of green synthesized Ag/ZnO NPs to control termite losses in peach were observed in both foliar and soil application. Overall Ag/ZnO NPs treatments showed significant results in foliar than in soil application.

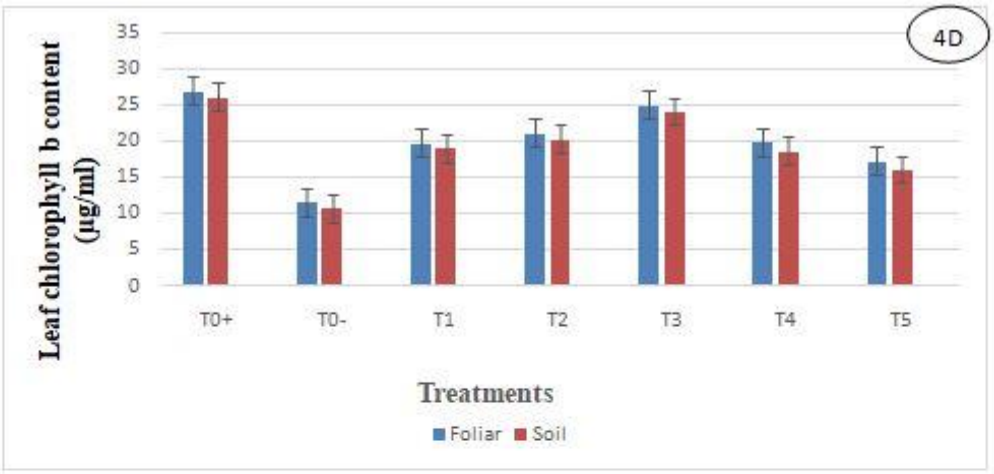
Analysis of data with reference to total leaf chlorophyll contents revealed differences in peach plants in response to foliar and soil application of insecticidal Ag/ZnO NPs treatments to control termite (Figure 4E). Significant decrease in total leaf chlorophyll contents were recorded (26.7 µg/ml) in foliar and (25.3 µg/ml) in soil. Best result found in

300ppm concentration (44.2 µg/ml) in foliar and (43.0 µg/ml) in soil application respectively. Decrease in total leaf chlorophyll contents were observed in higher as well in lower concentration of Ag/ZnO NPs treatments to control termite in peach in both foliar and soil applications. While comparing in total leaf chlorophyll contents showed better results (1.2 µg/ml) in foliar than soil application.






4C
Leaf chlorophyll a content (µg/ml)
35
30
25
20
15
10
5
0
T0+
T0-
T1
T2
T3
T4
T5
Treatments
Foliar
Soil


4D
Leaf chlorophyll b content (µg/ml)
35
30
25
20
15
10
5
0
T0+
T0-
T1
T2
T3
T4
T5
Treatments
Foliar
Soil

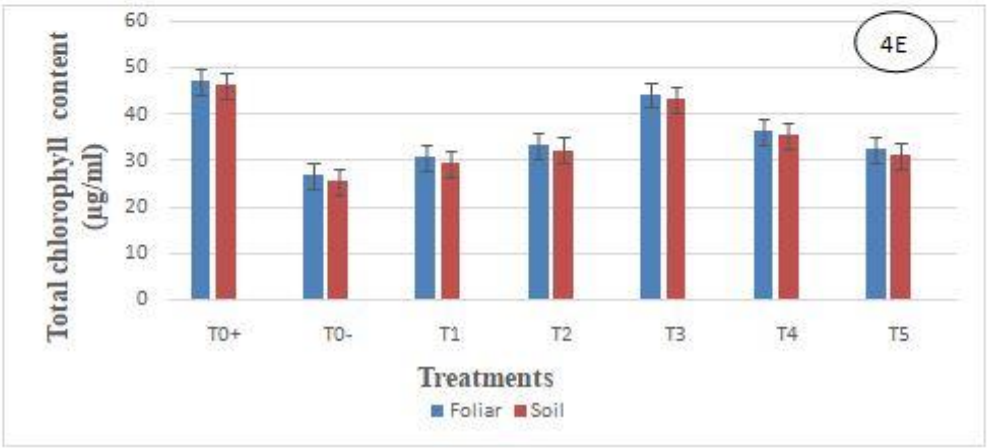

4E
Total chlorophyll content (µg/ml)
60
50
40
30
20
10
0
T0+
T0-
T1
T2
T3
T4
T5
Treatments
Foliar
Soil

Figure 4. (A) Role of nanocomposite Ag/ZnO on relative water content; **(B)** Role of nanocomposite Ag/ZnO on membrane stability index; **(C)** Role of nanocomposite Ag/ZnO on chlorophyll a content on peach ; **(D)** Role of nanocomposite Ag/ZnO chlorophyll b content on peach tree; **(E)** Role of nanocomposite Ag/ZnO on total chlorophyll a and b content on peach by controlling termites

3.3 Effect of Ag/ZnO on Biochemical Parameters on Peach in Response to Control Termites

Proline content is not increased in controlled plants under normal conditions such as environmental conditions exerts great impact on the production of proline in plants (Figure 5A). Increased proline content was observed in plants which treated with salt (28.2 µg/ml) in foliar and (27.7 µg/ml) in soil application as compared to control. Higher concentration of green synthesized nanoparticles gave increase level of prolein content in peach. Results of decreased prolein content observed at 300ppm (14.7 µg/ml) in foliar and (14.2 µg/ml) in soil application of Ag/ZnO NPs to control termite losses in peach.

Soluble sugar content was observed in peach plants and it increased in plants which treated with salt (10µg/ml) in foliar and (9.6 µg/ml) in soil application. When green synthesized nanoparticles were applied to treatments then soluble sugar content were decreased (Figure 5B). Reduced results were obtained in 300ppm (3.8µg/ml) in foliar and (3.6µg/ml) in soil. Protein content was observed in peach plants and there was increase in plant treated with salt (29.3µg/ml) in foliar and (28.8µg/ml) in soil application. When insecticidal Ag/ZnO nanocomposites applied to the treatment then protein content was raised (Figure 5C). Reduced results obtained in 300ppm (17.9µg/ml) in foliar and (17.7µg/ml) in soil. However significant results were obtained in both foliar and soil applications.

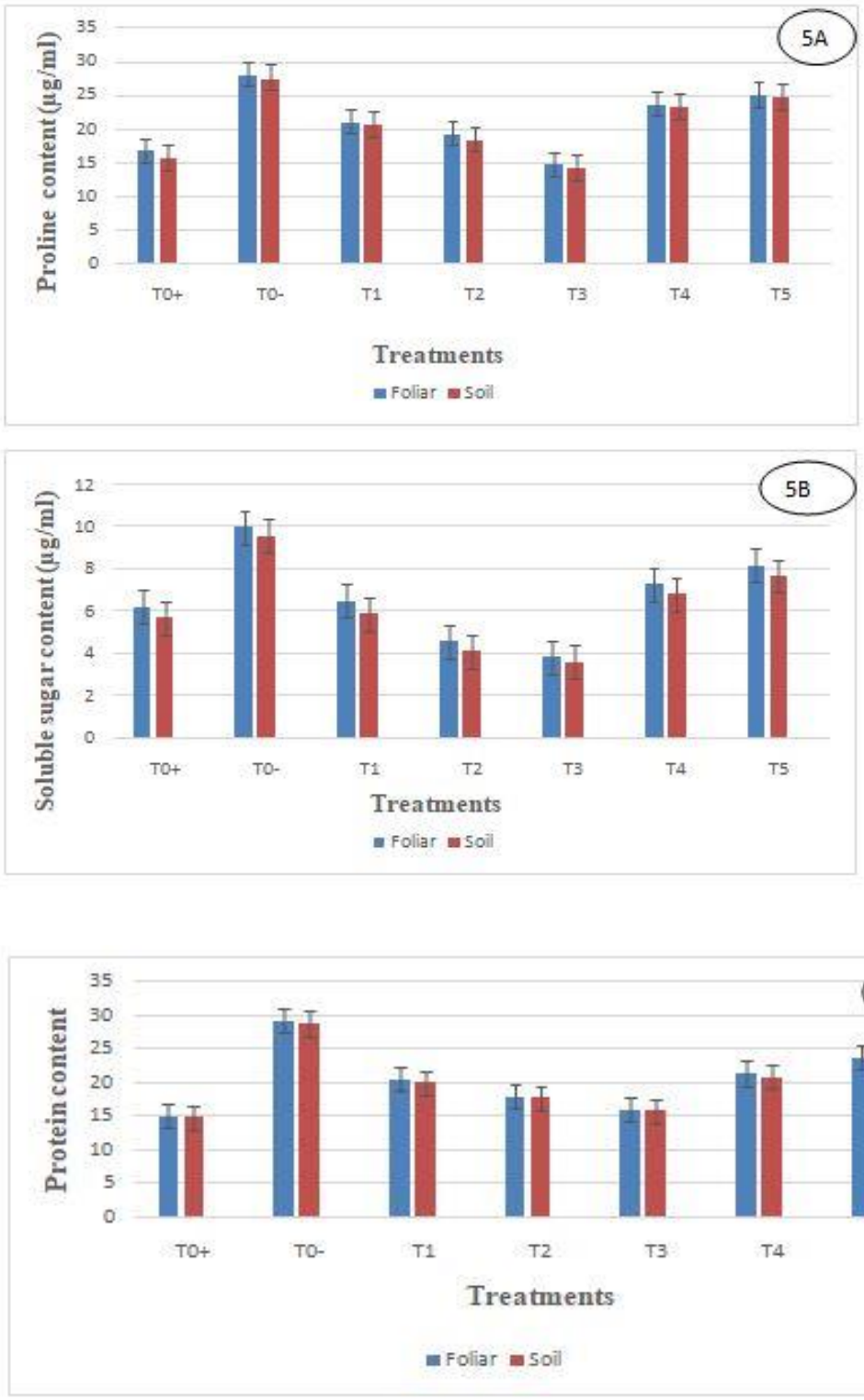


Figure 5. **(A)** Effect of Ag/ZnO on prolie content of peach tree to control termite; **(B)** Effect of Ag/ZnO on soluble sugar content of peach tree; **(C)** Effect of Ag/ZnO on protein content of peach tree;

Peach plants showed antioxidant activity of SOD in treatment plants (Figure 5D). There was increased activity of SOD in plants treated with silver zinc oxide salt (0.35nM/min/mg) in foliar and (0.33nM/min/mg) in soil application as compared to control. Application of silver zinc oxide nanoparticles decrease the production of antioxidant enzymes (SOD) while above optimum concentration there was increase in SOD production. Results of decreased production of SOD was recorded in 300ppm (0.19nM/min/mg) in foliar and (0.17nM/min/mg) in soil. Overall foliar application showed significant results in peach than soil application.

Peroxidase activity was observed higher in T0- which treated with silver zinc salt (19.7nM/min/mg) in foliar and (19.4nM/min/mg) in soil applications as compared to control. Results were decreased in 300ppm (9.4nM/min/mg) in foliar and (9nM/min/mg) in soil (Figure 5E). Overall foliar application showed better results than soil application of silver zinc oxide nanoparticles to control termite loss in peach. However, lower concentration decrease their activity.

Catalase activity was observed in peach when insecticidal developed silver zinc oxide nanoparticles applied to treatments. It was observed that in plants (T0-) treated with salt cause higher catalase activity (12.9nM/min/mg) in foliar and (12.7nM/min/mg) in soil applications. While decreased results obtained in 300ppm (6.8nM/min/mg) in foliar and (6.6) in soil application that shown in figure 5F.

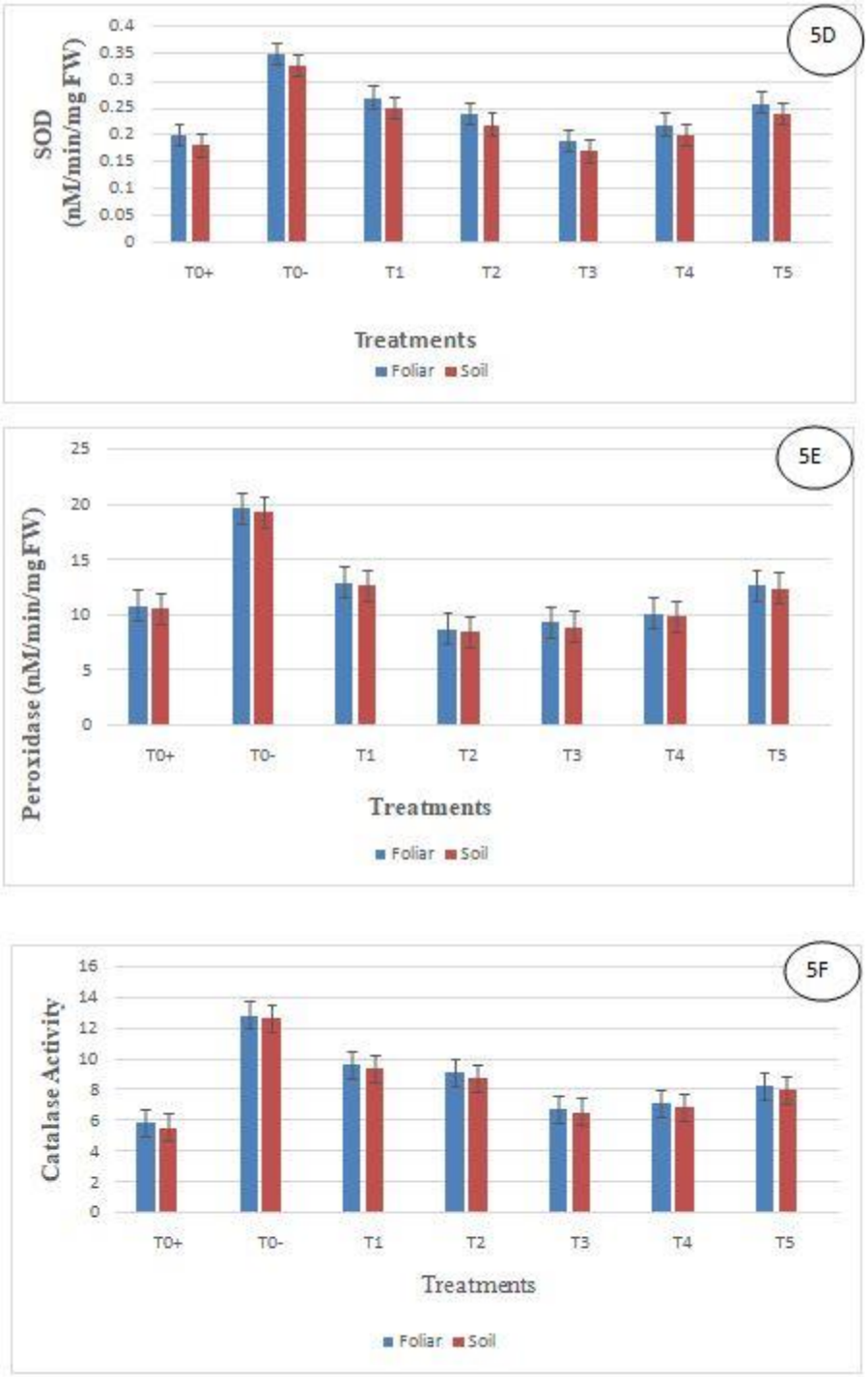


Figure 5. **(D)** Effect of Ag/ZnO nanocomposite on SOD of peach tree to control termite; **(E)** Effect of Ag/ZnO on POD of peach tree to control termite; **(F)** Effect of Ag/ZnO on catalase activity of peach tree to control termite;

4. Discussion

Nanotechnology has great potential to alter the present scenario of agriculture and with the help of innovative tools that made for the treatment of plant diseases, rapid pathogen identification using nano-based kits, boosting the ability of plants to absorb nutrients. NPs are ecofriendly and cheapest. Nanoparticles like silver, zinc, copper have major role in promoting the developmental stages of plants by alleviating the stress conditions or controlling insect pest attack o crops. Silver zinc oxides nanoparticles show effects on seed germinations, root, and shoot growth on species of rice (*Oryza sativa*) and Chinese cabbage (*Brassica campestris*), respectively. Ag/ZnO NPs has positive effect on plant germination and growth such as leaf area, chlorophyll content, phenolic content etc.

In present study Ag/ZnO NPs shows significant increase leaf area of peach plant. Laura et al. [22] reported that plants are affected with insects that put adverse effect on their growth and developmental stages and also draw nutrients from host (crops). Luo et al. [23] reported that insects not only produce damage on leaves in peach but also to branches and fruit i.e affect yield rate. In occurrence of bacterial spot on leaves of peach then initially water soaked lesions are produced. Leaf curl also occurred in peach by fungus *Taphrinadeformans.* It has adverse effect on leaves, shoot and fruit. Present works in accordance with [24, 25] who suggest that Zn and Ag NPs have great potential to improve plant growth and act as protective agent against insect pests. Their antimicrobial action have ability to eradicate plant pathogen growth without producing harmful effect on the neighboring plant tissues. Our fidings indicate that leaf area increased at 300ppm of Ag/ZnO NPs application.

Fresh and dry weight of plant were only affected with salt show decrease result as compared to control. Our finding represent that Ag/ZnO NPs at 300ppm show significant results in fresh and dry weight of leaf and Present work related to other studies. Yassin et al. [26]described that plants which treated with salt were remarkably reduce the leaves dry weight as compared to control plants. Salt stress susceptible plants were under developed due to less biomass and reduction occur in cell elongation and division with respect to wheat. Pincebourde et al. [27] who reported that when herbivorous insect feed crops, they produce greater effect on gaseous exchange. When aphids attack on plants then leaf surface, leaf dry weight were reduced. Similarly Mayer et al. [28] described that leaf feeding insect's i.e beetles or spittlebugs cause significant reduction in leaf growth. Ibrahim et al. [29]& Adil et al. [30], explained that ZnO

nanoparticles raised the bioavailability for crops that significantly increase leaf dry mass, root shoot dry matter and Ag NPs remarkably enhance fresh weight and dry weight in rice and give great potential to protect rice crop from bacterial infection and regulate growth of crop.

Reduction in relative water content was observed in plant that exposed to salt (Ag/ZnO). As relative water content has relation with water potential. Insects like aphids and thrips are plant feeding insects that cause declouration of plant leaf and also destroy entire plant. Leafhopper damage sycamore seedling cause stippling damage in leaf area and also reduce photosynthesis to two third. Kumar et al. [31] reported that relative water content were reduced in *Oenanthe javanica* cultivars with salt (NaCl) as compared to salt and our findings related to this study and previous studies gave confirmation about increment in relative water content due to NPs (Ag/ZnO). This research work represent remarkable results obtained at 300ppm Ag/ZnO concentration in peach plants.

Present study indicates that membrane stability index affected greatly with salt treated plants as compared to others. Work correlated with other studies as insect or bacteria entered in cell through membrane that effect stability and permeability. Application of Ag NPs at optimum level raised the stability of membrane and affect absorbency of membrane, nutrient uptake. More solute or ion leakage correlated to cell death (Koch et al. [32]& Martinez et al.[33].

Results recorded rise in photosynthetic pigment in a, b and I total leaf chlorophyll content is in response to treat optimum (300ppm) concentration of Ag/ZnO nanoparticles.our findings are related with(Rao et al.[34] stated that high plant growth obtained by the increasing chlorophyll quantity. Our findings are related with Racuciu&Creanga (2007) reported that chlorophyll content of maize crop achieved high by the optimum concentration of Ag/ZnO nanoparticles. Govorov & Carmeli [35] described that in photosynthetic system NPs produce chemical energy however high chlorophyll content gave high photosynthetic rate. Decrease level of chlorophyll is found in those leaves that were subjected to Ag/ZnO salt. Ashraf & Harris [36] reported that due to occurrence of leaves senescence injuries in thylakoid lamella or stoma, reduction in leaf area results in the inhibition of chlorophyll a, chlorophyll b biosynthesis.

In present scenario increase in proline concentration was measured in salt treated plants. Proline considered as stress adjuster. As it scavenger reactive oxygen species and maintain water balance in stress plant Malick, [37]. Whereas less amout of proline obtained when plants subjected with 300ppm Ag/ZnO nanoparticles. As increased Ag/ZnO NPs enhance the prolein production. Like higher concentration of Ag/ZnO nanoparticles gave higher proline quantity [15, 38]Our findings confirmed that proline was higly achieved in salt stressed plants.

In present study increased soluble sugar level was observed in plants that treated with Ag/ZnO salt and supports other studies. Soluble salts are one of important organic solutes that helps to keep homeostasis. Likewise in chickpea, production of soluble sugar increased by salt stress as related to previous study. Latif et al. (2017) describe that photosynthesis increased by the application of Ag/ZnO NPs and as a result net sugar content raised. Our findings are in accordance to the (Krishnaraj et al. 2012 & Jiang et al. 2012) who suggested that soluble sugar decreased at optimum concentrations of nanoparticles. Hassan et al. (2020) described that when higher application of Ag NPs treated plants gave higher prolein content as compared to control and our findings are co relate with it i.e higher Ag/ZnO nanoparticle produce high proline content.

Salama et al (2012) reported that high protein content was achieved at higher concentration of AgNPs concentration as compared to control in *Zea mays* and *Phaseolus vulgaris* and our findings are co related with this study as application of Ag/ZnO NPs at optimum concentration (300ppm), decrease protein contet however by increasing concentration protein content got increased. Mahawar et al (2018) explained that on cyanobacterium *Calothrix elenkinii,* higher concentration of silver and zinc nanoparticles gave remarkable high protein content (after the period of a week) as compared to control. From research work found that protein content raised in those plants that treated with silver zinc salt.

Results obtained from present work represent that increased concentration of superoxide dismutase in plants which subjected to silver zinc salt. The reason of increased SOD is due to anti-oxidant property of scavenging reactive oxygen species. As superoxide dismutase give defense against ROS. When peach plant subjected with salt SOD production was decreased. Krishnaraj et al. (2012) reported that at (300ppm) optimum concentration of Ag/ZnO decreases

the activity of superoxide dismutase.

Present work indicate that increased concentration of ascorbate peroxidase in plants which subjected to silver zinc salt. Because POD is involved in the detoxification of hydrogen peroxidase and another reason of increasing production of POD is due to anti-oxidant activity of scavenge ROS. AT 300ppm of Ag/ZnO nanoparticle application to plants, SOD production is decreased and our finding lined with others. Dimkpa et al. (2012) explained that higher concentration of Ag/ZnO nanoparticles increased the enzymatic activity of POD and SOD.

Catalase is a key factor that give protection against oxidative damage and it became triggered on the exposure of Ag/ZnO NPs in plants. Our findings are lined with Hassan et al (2020) explained that when castor seeds subjected to higher Ag/ZnO nanoparticles' concentration then CAT activity was increased and Nair et al. (2017) reported that in *Brassica napus* production of catalase activity was increased when plants treated with nanoparticles. Moreover Susmitha et al. (2019) nanoparticles helps a lot in controlling insect pest like zinc oxide, titanium oxide and aluminum oxide nanoparticles have great potential to control Sitophilus oryzae which cause harmful effect on stored grains or crops' seed.

This is having due to the possession of antimicrobial property that also protect from microbial infection. Present work give significant results in controlling termites on using the green synthesized silver zinc oxide nanoparticles in peach. Remarkable results obtained at 300ppm concentration of green synthesized Ag/ZnO NPs. Attia et al. (2015) explained that by using zinc oxide and aluminum oxide nanoparticles insect mortality rate enhanced with the increasing period of exposure and NPs' concentration and this present work correlate that termites controlled at optimum concentration of nanoparticles (300ppm) and also increased the plant growth factors like leaf area, chlorophyll content. Ahmadi et al. (2021) reported that when silver/ chitosan/polyethylene glycol nanocomposite were loaded with chlorpyrifos then significant results obtained at lower concentration of nanoparticles i.e it have effective result in controlling the existing insect pests and also have potential to kill the incoming insect and present research work supported other studies and give notable results in controlling termites and promoting the peach developmental stages like leaf area. Plants treated with at 300ppm green synthesized silver zinc oxides nanoparticles give suitable results in peach to control

termite and enhance growth factors

5. Conclusion and recommendation

It has been concluded from present work that green synthesized Ag/ZnO nanoparticles shown positive effect on peach plants to control termite. Termites are harmful for plants because they affect all developmental stages of plant. Many chemicals are used against insects but these agents prove harmful for environment and had adverse effect on the health of animals as well as humans. Nowadays biological methods are considered for controlling insects. Green synthesized Ag/ZnO nanoparticles used against termites is eco-friendly and nanoparticles help to maintain plant metabolism in stress condition. Nanotechnology helps to enhance the physiological, morphological and biochemical attributes in peach plants even in stress condition. To enhance the quality of peach, nanotechnology must applied at commercial level to avoid the environmental pollution, and health hazards produced by chemical agents.

Declaration:

Authors' Contributions: All authors of this research paper have contributed significantly to the literature study, writing, and methodology, and critically revised the review article. **ME**: Conceptualization/conceived the study idea, planned and designed the review structure, wrote the first draft of the manuscript, data validation, visualization, customized images, final draft **NIR.**: conceptualizations, suggestions. **FA**: helped in final draft editing and suggestions. All authors have read and agreed to the published version of the manuscript.

Funding: No funding available.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data collected or analyzed during this research are included in this article.

References

[1] A. Ullah, D. Khan, and S. Zheng, "The determinants of technical efficiency of peach growers: Evidence from Khyber Pakhtunkhwa, Pakistan," *Custos E Agronegocio Line,* vol. 13, pp. 211-238, 2017.

[2] A. Rehman, Z. Deyuan, I. Hussain, M. S. Iqbal, Y. Yang, and L. Jingdong, "Prediction of major agricultural fruits production in Pakistan by using an econometric analysis and

machine learning technique," *International Journal of Fruit Science,* vol. 18, no. 4, pp. 445-461, 2018.
[3] A. Hussain, S. Ali, M. Muhammad, W. Akram, S. M. Hussain, and K. Dawar, "Spatial distribution and risk associated with shot hole disease in apricot (Prunus armeniaca L.) in Northern Pakistan," *Archives of Phytopathology and Plant Protection,* vol. 56, no. 6, pp. 433-451, 2023.
[4] S. Razzaq *et al.*, "Cadmium stabilization and redox transformation mechanism in maize using nanoscale zerovalent-iron-enriched biochar in cadmium-contaminated soil," *Plants,* vol. 11, no. 8, p. 1074, 2022.
[5] S. Singh, "TERMITE TRAP: A NOVEL TECHNOLOGY FOR ECO-FRIENDLY MANAGEMENT OF TERMITES IN ORCHARDS," *Agricultural Research Journal,* vol. 57, no. 2, pp. 184-187, 2020.
[6] S. Govorushko, "Economic and ecological importance of termites: A global review," *Entomological Science,* vol. 22, no. 1, pp. 21-35, 2019.
[7] H. M. Salama, "Effects of silver nanoparticles in some crop plants, common bean (Phaseolus vulgaris L.) and corn (Zea mays L.)," *Int Res J Biotechnol,* vol. 3, no. 10, pp. 190-197, 2012.
[8] V. D. Rajput *et al.*, "Effect of nanoparticles on crops and soil microbial communities," *Journal of Soils and Sediments,* vol. 18, pp. 2179-2187, 2018.
[9] S. M. Zahedi, M. Karimi, and J. A. Teixeira da Silva, "The use of nanotechnology to increase quality and yield of fruit crops," *Journal of the Science of Food and Agriculture,* vol. 100, no. 1, pp. 25-31, 2020.
[10] A. Younas *et al.*, "Role of nanotechnology for enhanced rice production," *Nutrient dynamics for sustainable crop production,* pp. 315-350, 2020.
[11] K. A. Elsayed *et al.*, "Fabrication of ZnO-Ag bimetallic nanoparticles by laser ablation for anticancer activity," *Alexandria Engineering Journal,* vol. 61, no. 2, pp. 1449-1457, 2022.
[12] N. Maroufpour, M. Mousavi, B. Asgari Lajayer, and M. Ghorbanpour, "Biogenic Nanoparticles in the Insect World: Challenges and Constraints," *Biogenic Nano-Particles and their Use in Agro-ecosystems,* pp. 173-185, 2020.
[13] S. Mishra *et al.*, "Interaction mechanism of plant-based nanoarchitectured materials with digestive enzymes of termites as target for pest control: Evidence from molecular docking simulation and in vitro studies," *Journal of Hazardous Materials,* vol. 403, p. 123840, 2021.
[14] R. Sairam, "Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties," *Plant Growth Regulation,* vol. 14, pp. 173-181, 1994.
[15] A. Bernal-Vicente, D. Cantabella, J. Hernández, and P. Diaz-Vivancos, "The effect of mandelonitrile, a recently described salicylic acid precursor, on peach plant response against abiotic and biotic stresses," *Plant Biology,* vol. 20, no. 6, pp. 986-994, 2018.
[16] L. S. Bates, R. a. Waldren, and I. Teare, "Rapid determination of free proline for water-stress studies," *Plant and soil,* vol. 39, pp. 205-207, 1973.
[17] M. Dubois, K. Gilles, J. Hamilton, P. Robers, and F. Smith, "Determination of sugars," *Nature,* vol. 168, no. 4265, p. 167, 1951.

[18] N. Kresge, R. D. Simoni, and R. L. Hill, "The most highly cited paper in publishing history: Protein determination by Oliver H. Lowry," *Journal of Biological Chemistry,* vol. 280, no. 28, pp. e26-e28, 2005.
[19] D. Prochazkova, R. Sairam, G. Srivastava, and D. Singh, "Oxidative stress and antioxidant activity as the basis of senescence in maize leaves," *Plant Science,* vol. 161, no. 4, pp. 765-771, 2001.
[20] Y. Nakano and K. Asada, "Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts," *Plant and cell physiology,* vol. 22, no. 5, pp. 867-880, 1981.
[21] C. Parisi, M. Vigani, and E. Rodríguez-Cerezo, "Agricultural nanotechnologies: what are the current possibilities?," *Nano Today,* vol. 10, no. 2, pp. 124-127, 2015.
[22] B. Cariglino, P. Moisan, and M. B. Lara, "The fossil record of plant-insect interactions and associated entomofaunas in Permian and Triassic floras from southwestern Gondwana: A review and future prospects," *Journal of South American Earth Sciences,* vol. 111, p. 103512, 2021.
[23] C.-X. Luo, G. Schnabel, M. Hu, and A. De Cal, "Global distribution and management of peach diseases," *Phytopathology Research,* vol. 4, no. 1, pp. 1-15, 2022.
[24] A. M. Abdelaziz, S. S. Salem, A. M. Khalil, D. A. El-Wakil, H. M. Fouda, and A. H. Hashem, "Potential of biosynthesized zinc oxide nanoparticles to control Fusarium wilt disease in eggplant (Solanum melongena) and promote plant growth," *BioMetals,* vol. 35, no. 3, pp. 601-616, 2022.
[25] M. Tariq, K. N. Mohammad, B. Ahmed, M. A. Siddiqui, and J. Lee, "Biological synthesis of silver nanoparticles and prospects in plant disease management," *Molecules,* vol. 27, no. 15, p. 4754, 2022.
[26] M. Yassin *et al.*, "Comparative performance of two bread wheat (Triticum aestivum L.) genotypes under salinity stress," *Applied Ecology & Environmental Research,* vol. 17, no. 2, 2019.
[27] S. Pincebourde and J. Ngao, "The impact of phloem feeding insects on leaf ecophysiology varies with leaf age," *Frontiers in Plant Science,* vol. 12, p. 625689, 2021.
[28] M. C. Martínez-Ballesta, L. López-Pérez, B. Muries, O. Muñoz-Azcarate, and M. Carvajal, "Climate change and plant water balance: the role of aquaporins–a review," *Climate Change, Intercropping, Pest Control and Beneficial Microorganisms: Climate change, intercropping, pest control and beneficial microorganisms,* pp. 71-89, 2009.
[29] E. Ibrahim *et al.*, "Biosynthesis of silver nanoparticles using endophytic bacteria and their role in inhibition of rice pathogenic bacteria and plant growth promotion," *RSC advances,* vol. 9, no. 50, pp. 29293-29299, 2019.
[30] M. Adil *et al.*, "Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress," *Frontiers in plant science,* vol. 13, p. 932861, 2022.
[31] S. Kumar *et al.*, "Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (Oenanthe javanica) cultivars," *Frontiers in plant science,* vol. 12, p. 660409, 2021.
[32] S. Kumar, V. Singh, and A. Tanwar, "Structural, morphological, optical and photocatalytic properties of Ag-doped ZnO nanoparticles," *Journal of Materials Science: Materials in Electronics,* vol. 27, pp. 2166-2173, 2016.

[33] S. Mathur, D. Agrawal, and A. Jajoo, "Photosynthesis: response to high temperature stress," *Journal of Photochemistry and Photobiology B: Biology,* vol. 137, pp. 116-126, 2014.
[34] S. Rao, A. Qayyum, A. Razzaq, M. Ahmad, I. Mahmood, and A. Sher, "Role of foliar application of salicylic acid and l-tryptophan in drought tolerance of maize," *J. Anim. Plant Sci,* vol. 22, no. 3, pp. 768-772, 2012.
[35] A. O. Govorov and I. Carmeli, "Hybrid structures composed of photosynthetic system and metal nanoparticles: plasmon enhancement effect," *Nano letters,* vol. 7, no. 3, pp. 620-625, 2007.
[36] M. Ashraf and P. J. Harris, "Photosynthesis under stressful environments: An overview," *Photosynthetica,* vol. 51, pp. 163-190, 2013.
[37] H. Mahawar, R. Prasanna, S. B. Singh, and L. Nain, "Influence of silver, zinc oxide and copper oxide nanoparticles on the cyanobacterium Calothrix elenkinii," *BioNanoScience,* vol. 8, pp. 802-810, 2018.
[38] M. N. Helaly, M. A. El-Metwally, H. El-Hoseiny, S. A. Omar, and N. I. El-Sheery, "Effect of nanoparticles on biological contamination of'in vitro'cultures and organogenic regeneration of banana," *Australian Journal of Crop Science,* vol. 8, no. 4, pp. 612-624, 2014.