

## Research Article

# Physiological and biochemical responses of chickpea (*Cicer arietinum*) to endophytic fungi and silver nanoparticles in relation to *Helicoverpa armigera*

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## Abstract

Chickpea (*Cicer arietinum* L.), an important pulse crop of the Fabaceae family, is widely cultivated due to its high protein content and nutritional value. In Pakistan, major chickpea-growing regions include the Thal desert, Layyah, Bhakkar, Chowk Azam, and Chobara. However, chickpea production is severely constrained by insect pests, particularly *Helicoverpa armigera* (cotton bollworm), a destructive chewing pest that damages stems, delays crop growth, and reduces yield potential. Sustainable and eco-friendly approaches to pest control are therefore essential. In the present study, green-synthesized silver nanoparticles (AgNPs) in combination with *Metarhizium anisopliae* were investigated for their physiological, biochemical, and pesticidal effects on chickpea under controlled conditions. Chickpea plants were treated with two concentrations of AgNO<sub>3</sub> (5% and 10%) in combination with *M. anisopliae* (2% and 4%) through foliar application prior to pest infestation. The ingestion of AgNPs by *H. armigera* larvae caused gut damage and subsequent mortality, thereby protecting the plants. Mortality rates increased with both concentration and exposure time, reaching 20%, 40%, 60%, and 80% after 24, 48, 72, and 96 hours, respectively. In addition to pest suppression, treated plants exhibited significant improvements in physiological and biochemical attributes. Biomass accumulation, root length, and shoot height were enhanced, while photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) showed a marked increase compared to controls. Furthermore, biochemical assays revealed elevated levels of reactive oxygen species (MDA and H<sub>2</sub>O<sub>2</sub>) alongside enhanced activities of enzymatic antioxidants (SOD, POD, CAT) and non-enzymatic antioxidants (TSP), indicating activation of the plant defense system. The study, conducted in a completely randomized design with three replicates, demonstrates that the synergistic application of silver nanoparticles and *M. anisopliae* offers a promising, safe, and environmentally sustainable strategy for managing *H. armigera* infestation in chickpea while simultaneously promoting plant growth and physiological performance.

**Keywords:** Chickpea (*Cicer arietinum*), *Helicoverpa armigera*, Silver nanoparticles (AgNPs), *Metarhizium anisopliae*, Antioxidant defense system, Physiobiochemical response

## Introduction

Chickpea (*Cicer arietinum* L.) is one of the earliest domesticated pulse crops and holds an important place in global agriculture as a key source of plant-based protein. Belonging to the Fabaceae family, it is cultivated extensively in over 50 countries and ranks as the third most important legume after dry beans and peas (Kumar *et al.*, 2018; Gowda *et al.*, 2019). Historically, it was one of the seven founding crops domesticated in the Fertile Crescent and has since spread across diverse agro-ecological zones (Jha & Warkentin, 2020). In Pakistan, chickpea is the most widely grown pulse crop, with nearly 2.2 million acres dedicated to its cultivation, particularly in the rainfed Thal desert regions of Layyah, Bhakkar, and Chobara (Abbas *et al.*, 2021). Despite its nutritional and economic value, Pakistan has some of the lowest chickpea yields in the world and is often forced to rely on imports from Canada, Turkey, and Australia to meet domestic demand (Maurya & Kumar, 2018). Nutritionally, chickpea is highly regarded for its high protein content (25-30%), essential amino acids such as lysine and tryptophan, dietary fiber, and bioactive compounds including vitamins and minerals (Dhankhar *et al.*, 2019). Its high lysine levels complement cereal-

based diets that are deficient in this amino acid, making it a cornerstone for addressing malnutrition in resource-poor communities (Grewal *et al.*, 2020). Moreover, chickpea consumption has been associated with reduced risks of cardiovascular disease, diabetes, and some forms of cancer (Messina, 2014). Beyond nutritional contributions, chickpea plays a critical role in sustainable agriculture by fixing atmospheric nitrogen, enriching soil fertility, and reducing the need for chemical fertilizers (Patra *et al.*, 2016; Kaur *et al.*, 2022). Thus, chickpea stands at the intersection of nutritional security, economic livelihoods, and sustainable agricultural practices.

Despite its importance, chickpea production is significantly constrained by abiotic and biotic stresses, which collectively limit productivity and threaten its global availability. Abiotic stresses such as drought, heat, poor soil fertility, and salinity severely impact growth, nodulation, and nitrogen fixation, reducing yield potential across rainfed regions. For example, salinity alone affects nearly 40% of the world's arable land and has been reported to suppress chickpea productivity by impairing root development and nutrient uptake. In South Asia, where chickpea is primarily cultivated in marginal environments, these stresses

are particularly severe and often coincide with biotic challenges. Among biotic factors, insect pests represent the most damaging constraints, with *Helicoverpa armigera* recognized as the most destructive pest of chickpea worldwide (Dinesh *et al.*, 2017). This polyphagous insect feeds on over 182 plant species, including cotton, maize, tomato, and soybean, but causes the greatest damage to chickpea by attacking flowers and pods, leading to direct yield losses (Fathipour & Sedaratian, 2013; Ramana *et al.*, 2022). Its high fecundity, polyphagy, and adaptability have allowed *H. armigera* to spread across Asia, Africa, and Australia, where it accounts for millions of dollars in annual agricultural losses (Kriticos *et al.*, 2015). Conventional management of this pest relies heavily on synthetic insecticides, but their overuse has led to widespread resistance development, environmental pollution, disruption of beneficial insect populations, and risks to human health (Ngai & McDowell, 2017; Kapinder *et al.*, 2021). The persistent challenges of pesticide resistance and ecological harm highlight the urgent need for sustainable, eco-friendly pest management solutions for chickpea cultivation.

One promising alternative approach to chemical pesticides is the use of nanotechnology in agriculture. Silver nanoparticles (AgNPs) have emerged as a particularly effective tool due to their broad-spectrum antimicrobial, antifungal, and insecticidal properties. AgNPs can be synthesized through physical, chemical, or biological means, but green synthesis using plants, fungi, or microbes is increasingly favored because it is cost-effective, rapid, and environmentally safe (Dobrucka & Długaszewska, 2015; Haq *et al.*, 2020). In green synthesis, phytochemicals such as flavonoids, terpenoids, and organic acids act as natural reducing and stabilizing agents, producing nanoparticles of diverse morphologies without harmful byproducts (Yadav *et al.*, 2014). These nanoparticles exhibit unique physicochemical properties, including small size and large surface area, which enhance their reactivity and bioactivity (Abdel-Aziz *et al.*, 2014). In plants, foliar applications of AgNPs have been shown to enhance physiological and biochemical processes, including improved chlorophyll content, photosynthetic efficiency, antioxidant enzyme activity, and biomass accumulation (Abbas *et al.*, 2021; Ma *et al.*, 2021). Against insect pests such as *H. armigera*, AgNPs exert toxic effects by accumulating in the gut, disrupting digestive processes, damaging cellular membranes, and ultimately causing mortality (Ur Rahim *et al.*, 2021). Importantly, their application in pest management is considered relatively safe for humans and the environment, making AgNPs a promising component of integrated pest management (IPM) systems.

Complementary to nanotechnology, endophytic and entomopathogenic fungi offer another sustainable method of pest control. Endophytic fungi inhabit plant tissues without causing disease and are known to enhance plant growth, stress tolerance, and defense against pests through the production of bioactive metabolites (Dutta *et al.*, 2014; Uzma *et al.*, 2019). Entomopathogenic fungi such as *Metarhizium anisopliae* infect insects by penetrating the

cuticle, proliferating inside the host, and releasing toxins that eventually lead to death (Erler & Ates, 2015; Zhao *et al.*, 2016). These fungi have demonstrated high effectiveness against a variety of pests while posing minimal risks to non-target organisms and the environment. In chickpea systems, *M. anisopliae* has been identified as a potential biocontrol agent against *H. armigera*, offering a natural, eco-friendly alternative to chemical insecticides (Sinno *et al.*, 2020). Beyond direct insecticidal action, endophytic fungi can enhance the physiological status of plants by promoting growth, increasing nutrient uptake, and stimulating defense pathways through the induction of antioxidants and secondary metabolites (Khan *et al.*, 2018). This dual action—direct pest suppression and indirect plant defense enhancement—makes fungal biocontrol agents an attractive strategy in integrated pest management.

Recent advances suggest that combining nanotechnology with fungal biocontrol can yield synergistic effects in pest management. For instance, the application of AgNPs alongside *M. anisopliae* has been reported to increase insect mortality rates more effectively than either agent alone (Yan *et al.*, 2018; Raguvaran *et al.*, 2021). AgNPs may enhance the infectivity of fungi by weakening insect defenses, thereby facilitating fungal penetration and proliferation. Simultaneously, both agents can stimulate host plant defenses, leading to elevated levels of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as non-enzymatic antioxidants like total soluble proteins (TSP) (Acharya & Pal, 2020; Abbas *et al.*, 2021). This combined approach not only ensures higher pest mortality but also promotes better plant growth and resilience under biotic stress. In chickpea, such integrated nanobiotechnological strategies hold particular promise, given the crop's vulnerability to *H. armigera* and the limitations of chemical-based pest control. By reducing dependence on synthetic pesticides, these methods contribute to environmentally sustainable agriculture while safeguarding crop productivity.

In light of the rising global demand for pulses and the increasing challenges posed by abiotic and biotic stresses, it is essential to develop innovative, eco-friendly strategies for chickpea protection. The use of green-synthesized AgNPs in combination with endophytic fungi such as *M. anisopliae* represents a promising frontier in sustainable pest management. These approaches not only directly suppress *H. armigera* populations but also enhance the physiological and biochemical responses of chickpea, including photosynthetic efficiency, antioxidant activity, and biomass production. Given the crop's nutritional, economic, and ecological importance, improving chickpea resilience through nanobiotechnology and biological control is vital for ensuring food and nutritional security, particularly in regions heavily dependent on pulses such as South Asia. The present study therefore aims to evaluate the physiological and biochemical responses of chickpea to treatments with silver nanoparticles and endophytic fungi in relation to *H. armigera* infestation, providing insights into integrated strategies for sustainable chickpea production.

## Materials and methods

### Experimental site and insect collection

The experiment was conducted at the Department of Entomology, University of Agriculture Faisalabad (UAF), Pakistan. A chickpea (*Cicer arietinum* L.) variety CP1 was used for the study. Seeds were treated with the endophytic fungus *Metarhizium anisopliae* prior to sowing. Third- and fourth-instar larvae of *Helicoverpa armigera* were collected from cotton fields at the Entomological Research Institute, UAF. To prevent cannibalism, larvae were maintained individually in Petri dishes under controlled laboratory conditions (25±2 °C, 70±5% RH, and 12:12 h light: dark cycle) and reared on an artificial diet.

### Seed sowing and preparation of neem leaf extract

Fungus-treated chickpea seeds were sown in pots under greenhouse conditions. Standard agronomic practices were followed throughout the growth period to maintain plant health. Fresh neem (*Azadirachta indica*) leaves were collected from the Entomology Department, UAF, and thoroughly washed with distilled water to remove surface contaminants. Leaves were shade-dried for 3-4 h, ground into powder, and 18 g of powdered leaves were extracted in 100 mL double-distilled water. The mixture was boiled at 70 °C for 30 min, cooled to room temperature, and filtered through Whatman No.1 filter paper. The extract was stored at 4 °C until use for the synthesis of silver nanoparticles (AgNPs).

### Green synthesis of silver nanoparticles

Silver nanoparticles were synthesized using neem leaf extract as a reducing and stabilizing agent. A 1 mM AgNO<sub>3</sub> solution was prepared by dissolving 169 mg AgNO<sub>3</sub> in 100 mL distilled water. Ten milliliters of neem extract were added to 90 mL of AgNO<sub>3</sub> solution and heated with continuous stirring for 1 h. A change in solution color indicated the formation of AgNPs, which were stored for subsequent applications.

### Characterization of silver nanoparticles

The biosynthesis of AgNPs was confirmed by ultraviolet–visible (UV-Vis) spectroscopy using a spectrophotometer in the range of 200-800 nm, based on the characteristic surface plasmon resonance (SPR) peaks of AgNPs.

### Insect rearing

*H. armigera* larvae were reared on an artificial diet prepared using wheat flour, cornmeal, soybean meal, dried yeast, brewer's yeast, calcium carbonate, vitamins/mineral supplements, agar powder, honey, and distilled water. The diet was sterilized, cooled, and dispensed into Petri dishes for larval feeding.

## Application of treatments

Two concentrations of AgNPs (5% and 10%) were applied as foliar sprays, while *M. anisopliae* (2%) was applied as a soil drench. Treatments included:

- AgNPs alone (5% and 10%),
- *M. anisopliae* alone (2%),
- Combination of AgNPs (5% and 10%) with *M. anisopliae* (2%),
- Control (distilled water).

### Larvicidal bioassay

To assess larvicidal activity, third- and fourth-instar *H. armigera* larvae were fed with chickpea leaves and pods treated with different nanoparticle and fungal applications. Mortality was recorded at 24, 48, 72, and 96 h post-feeding.

### Morphological parameters

Plant growth responses were evaluated by recording shoot length (cm), root length (cm), fresh and dry weights of shoots and roots (g). Dry weights were obtained by oven-drying samples at 70 °C until constant weight was achieved.

### Physiological parameters

Photosynthetic pigments were estimated following Harborne (1984). Fresh leaves (500 mg) were homogenized in 80% acetone and centrifuged. Absorbance was measured at 663, 646, and 470 nm, and chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated using standard equations.

### Biochemical and antioxidant enzyme assays

Leaf samples were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA, and centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was used for enzyme assays. Protein concentration was determined using bovine serum albumin as standard. Catalase (CAT) Activity was measured by monitoring the decomposition of H<sub>2</sub>O<sub>2</sub> at 240 nm. Peroxidase (POD) Activity was determined using guaiacol as substrate, measuring absorbance at 470 nm. Superoxide dismutase (SOD): Activity was assayed by inhibition of photochemical reduction of nitro-blue tetrazolium (NBT). Total soluble proteins (TSP): Quantified using standard protocols. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA): Estimated as markers of oxidative stress.

## Results

### Morphological study

#### Shoot fresh weight

Application of different concentrations of silver nanoparticles and *M. anisopliae* significantly influenced the shoot fresh weight of chickpea plants. The maximum

shoot fresh weight (44.10 g) was recorded in treatment T2 (10% Ag + 2% F), followed by T4 (10% Ag alone), compared to the control. These findings indicate that higher concentrations of AgNPs, particularly when combined with fungal treatment, enhanced shoot biomass accumulation (Figure 1a).

*Root fresh weight*

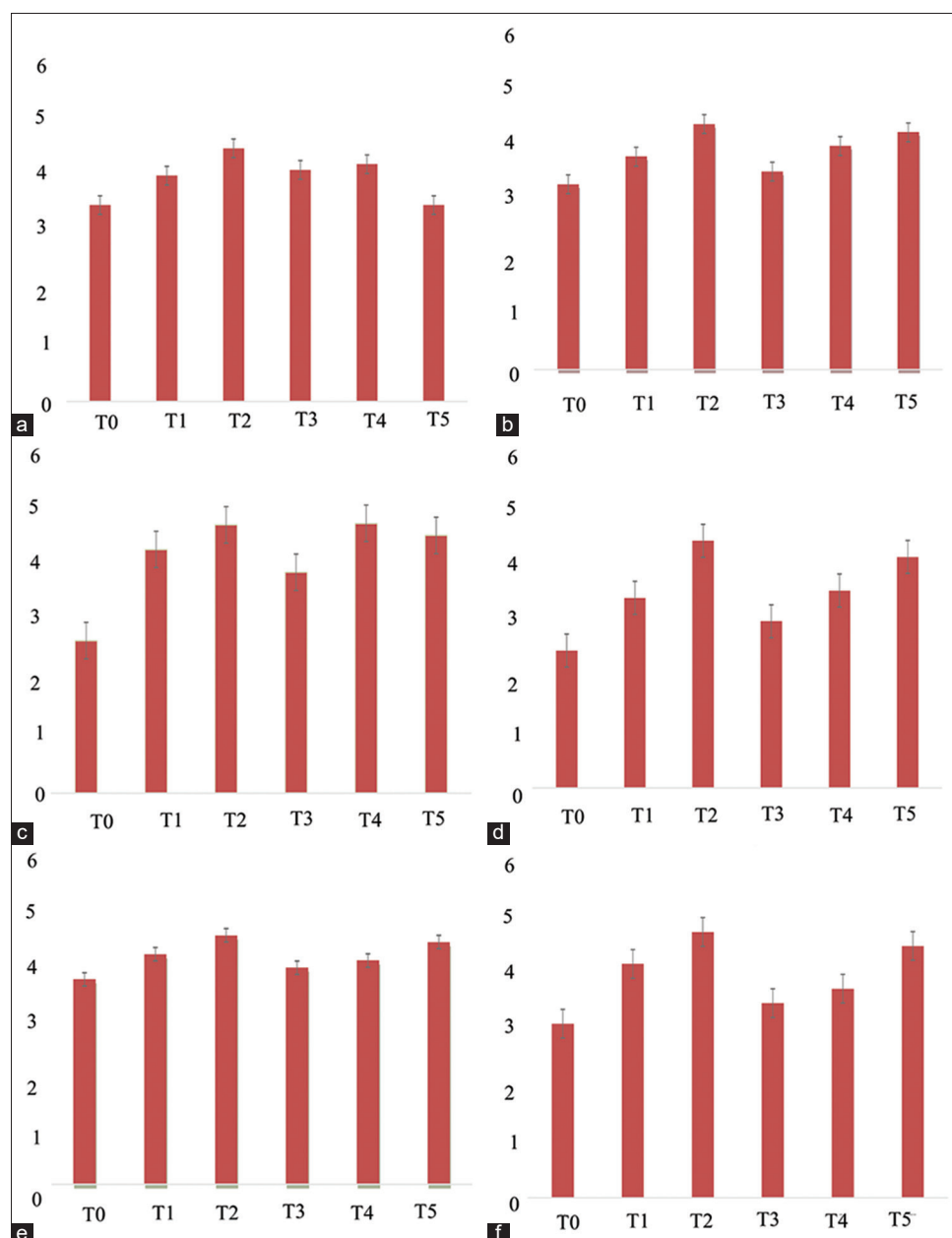
Root fresh weight of chickpea was also significantly enhanced under different treatments. The highest root fresh weight (3.23 g) was observed in T2 (10% Ag + 2% F), followed by T4 (10% Ag), when compared with the control. These results suggest that the synergistic effect of nanoparticles and fungal inoculation promoted improved root growth, which may contribute to better nutrient and water uptake (Figure 1b).

*Shoot dry weight*

Significant variations were also observed in shoot dry weight among treatments. The maximum shoot dry weight (5.13 g) was recorded in T4 (10% Ag), followed by T2 (10% Ag + 2% F). These findings highlight that AgNPs not only improve fresh biomass but also contribute to greater dry matter accumulation in chickpea shoots (Figure 1c).

*Root dry weight*

Root dry weight was significantly affected by nanoparticle and fungal treatments. The highest root dry weight (0.56 g) was recorded in T2 (10% Ag + 2% F), followed by T5 (2% F), in comparison to the control. This improvement in root dry biomass under combined



**Figure 1:** Summarize the morphological characteristics of the plant 10% silver nanoparticles increased as compared to 5% silver nanoparticles. T0 = Control, T1 = 5% Ag + 2% F, T2 = 10% Ag + 2% F, T3 = 5% Ag, T4 = 10% Ag, T5 = 2% F, Ag = Silver NPs and F = Fungus (*Metarhizium anisopliae*)

treatments suggests enhanced plant vigor and better root system development (Figure 1d).

#### Shoot length

Shoot length of chickpea plants varied significantly with the application of different treatments. The longest shoots (39.3 cm) were recorded under T2 (10% Ag + 2% F), followed by T5 (2% F), whereas the shortest shoots were observed in the control. These results demonstrate that the combined application of AgNPs and *M. anisopliae* enhanced plant height, reflecting improved vegetative growth (Figure 1e).

#### Root length

Root length was also significantly enhanced under nanoparticle and fungal treatments. The maximum root length (17.2 cm) was noted in T2 (10% Ag + 2% F), followed by T5 (2% F), compared to the control. The substantial increase in root length under combined treatments indicates a strong positive impact on below-ground plant development, likely supporting improved water and nutrient acquisition (Figure 1f).

### Physiological study

#### Chlorophyll a

Application of different concentrations of silver nanoparticles (AgNPs) and *Metarhizium anisopliae* significantly enhanced the chlorophyll a content of chickpea plants. The maximum value (1.83 mg g<sup>-1</sup> FW) was recorded under T2 (10% Ag + 2% F), followed by T5 (2% F), whereas the control (T0) showed the lowest values. (Figure 2a) further illustrates that the combined application of 10% AgNPs and 2% fungus resulted in a considerable increase compared to 5% AgNPs and control, confirming the strong positive impact of the nanoparticle-fungal treatment on chlorophyll a synthesis.

#### Chlorophyll a/b Ratio

The ratio of chlorophyll a to chlorophyll b, which reflects the balance and efficiency of the light-harvesting complex, was also significantly influenced by treatments. The maximum value (3.68) was observed in T2 (10% Ag + 2% F), followed by T5, while the control plants had the lowest ratio. The graphical presentation (Figure 2b) clearly shows that the combined nanoparticle and fungal treatment produced the greatest enhancement in chlorophyll a/b ratio compared to other treatments.

#### Chlorophyll b

A significant improvement in chlorophyll b was also observed in response to nanoparticle and fungal treatments. The highest value (0.062 mg g<sup>-1</sup> FW) was measured in T2 (10% Ag + 2% F), followed by T5 (2% F), whereas the lowest pigment levels were found in untreated control plants. The corresponding graphical trend (Figure 2c)

revealed that higher concentrations of AgNPs, especially when combined with fungus, produced a more pronounced effect compared to 5% AgNPs alone.

#### Carotenoids

Carotenoid content was significantly affected by the treatments, showing maximum accumulation under T2 (0.85 mg g<sup>-1</sup> FW), followed by T5, while the lowest content was observed in the control. Carotenoids, which act as essential photoprotective pigments, were strongly enhanced by silver nanoparticles in combination with fungal inoculation. Graphical data (Figure 2d) confirmed that T2 had the strongest effect in promoting carotenoid biosynthesis compared to other treatments.

#### Total chlorophyll

Total chlorophyll content followed a similar trend, with T2 (10% Ag + 2% F) recording the maximum value (2.69 mg g<sup>-1</sup> FW), followed by T5 (2% F). Control plants exhibited the lowest pigment levels, highlighting the enhancing effect of nanoparticles and fungi on chlorophyll biosynthesis. As depicted in (Figure 2e) total chlorophyll accumulation was greatest in the combined nanoparticle-fungal treatment, further emphasizing the synergistic effect of AgNPs and *M. anisopliae*.

### Summary of physiological study

The results of the physiological study revealed that silver nanoparticles and *M. anisopliae* significantly improved photosynthetic pigment concentrations in chickpea plants. Across all measured parameters—chlorophyll a, chlorophyll b, carotenoids, total chlorophyll, and chlorophyll a/b ratio—the combined treatment T2 (10% Ag + 2% F) consistently showed the highest values, followed by T5 (2% F). These findings indicate that the synergistic effect of nanoparticles and fungi enhanced pigment biosynthesis, stabilized photosynthetic machinery, and improved overall plant physiological performance.

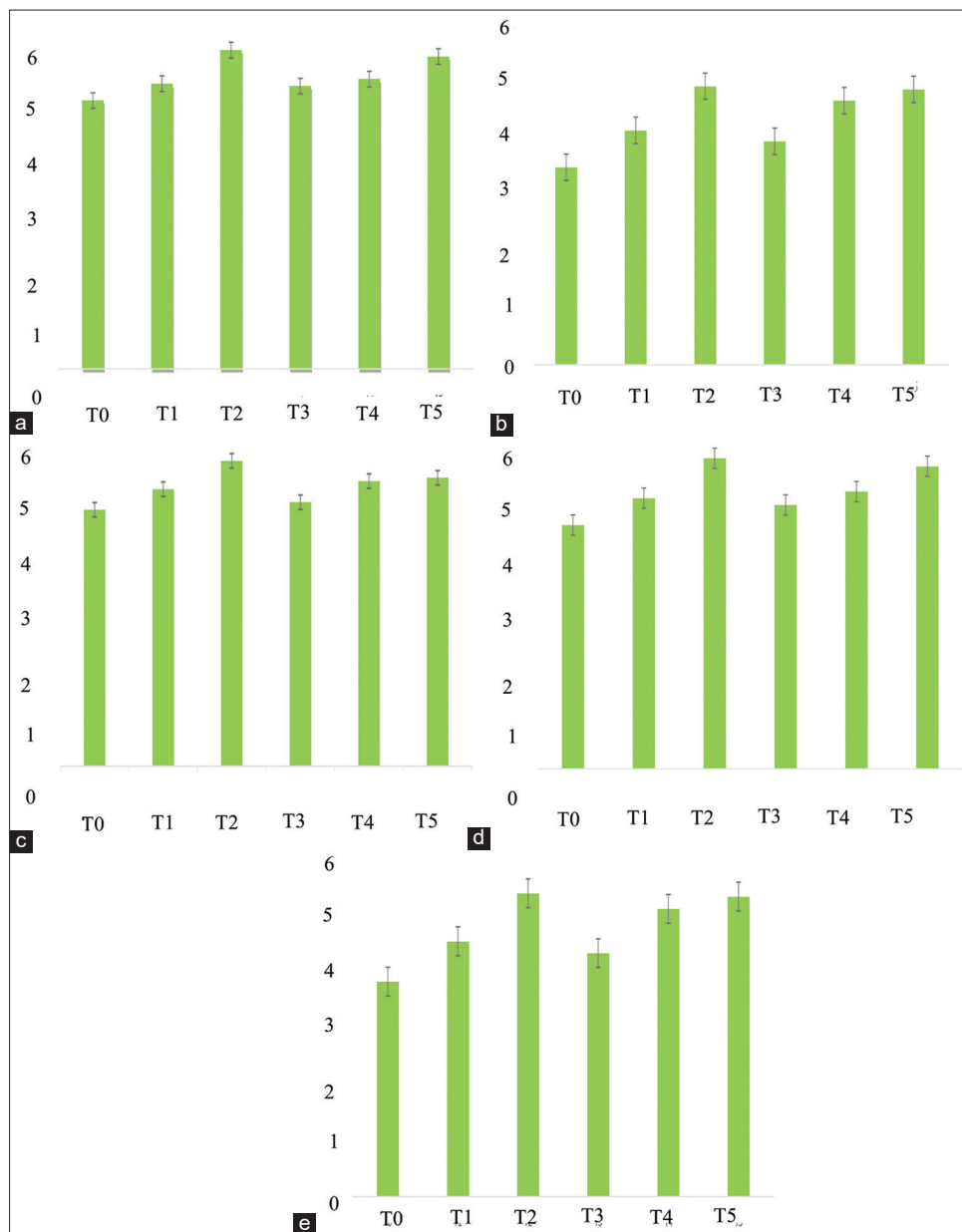
### Enzymatic study

#### Catalase (CAT)

Application of different concentrations of silver nanoparticles (AgNPs) and *Metarhizium anisopliae* significantly influenced catalase activity in chickpea plants. The maximum catalase activity (0.050 mg<sup>-1</sup>) was observed in treatment T2 (10% Ag + 2% F), followed by T5 (2% F), whereas the control (T0) recorded the lowest values. (Figure 3a) further supports these findings, showing that catalase activity increased progressively with higher nanoparticle concentration, especially in combination with fungal inoculation.

#### Peroxidase (POD)

Peroxidase activity was significantly enhanced by nanoparticle and fungal treatments. The highest value



**Figure 2:** (a-e) Summarize the physiological characteristics of the plant 10% silver nanoparticles increased as compared to 5% silver nanoparticles. T0 = Control, T1 = 5% Ag + 2% F, T2 = 10% Ag + 2% F, T3 = 5% Ag, T4 = 10% Ag, T5 = 2% F, Ag = Silver NPs and F = Fungus (*Metarhizium anisopliae*)

(25.30 mg<sup>-1</sup>) was recorded in T2 (10% Ag + 2% F), followed by T5 (2% F), while the control exhibited minimal activity. The graphical data (Figure 3b) illustrate this upward trend, highlighting the synergistic role of AgNPs and fungal inoculation in enhancing peroxidase levels.

**Superoxide dismutase (SOD)**

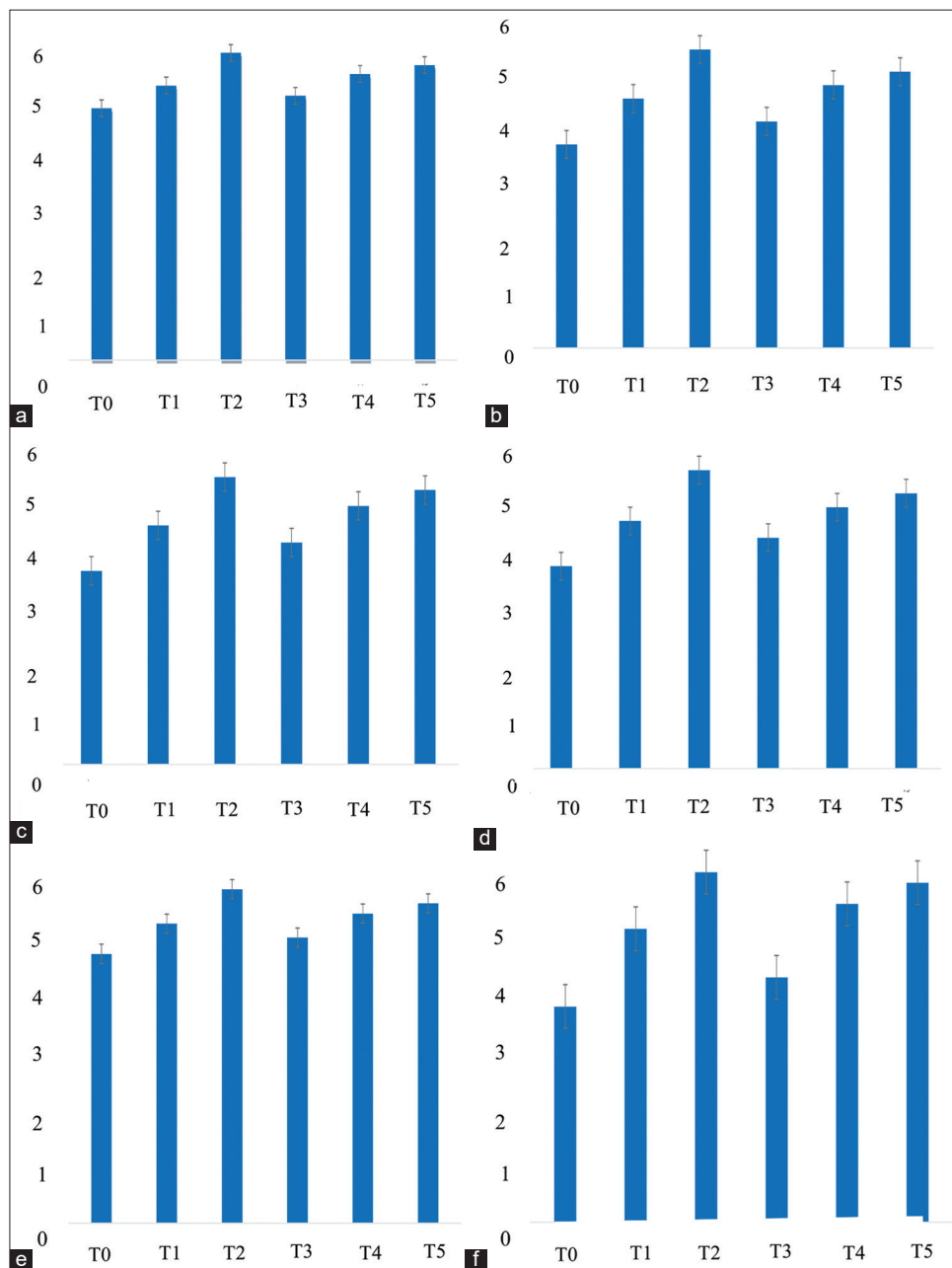
Superoxide dismutase activity followed a similar pattern, with the maximum value (24.92 mg<sup>-1</sup>) observed in T2 (10% Ag + 2% F). T5 (2% F) also showed a considerable increase compared to the control. (Figure 3c) clearly depicts that higher nanoparticle concentration in combination with fungal inoculation significantly boosted SOD activity, reflecting improved oxidative stress regulation in treated plants.

**Total soluble proteins (TSP)**

Significant increases in total soluble protein content were recorded under nanoparticle and fungal treatments. The maximum value (25.72 mg g<sup>-1</sup> FW) was observed in T2 (10% Ag + 2% F), followed by T5 (2% F). (Figure 3d) illustrates this improvement, demonstrating the combined role of AgNPs and fungi in promoting protein metabolism and overall plant vigor.

**Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**

Hydrogen peroxide content also showed significant variation among treatments. The highest value (0.06 mg g<sup>-1</sup> FW) was recorded in T2 (10% Ag + 2% F), followed by T5 (2% F). Control plants showed the lowest



**Figure 3:** (a-f) Summarize the enzymatic activity of the plant 10% silver nanoparticles increased as compared to 5% silver nanoparticles. T0 = Control, T1 = 5% Ag + 2% F, T2 = 10% Ag + 2% F, T3 = 5% Ag, T4 = 10% Ag, T5 = 2% F, Ag = Silver NPs and F = Fungus (*Metarhizium anisopliae*)

H<sub>2</sub>O<sub>2</sub> content. The results (Figure 3e) suggest that AgNPs in combination with fungal inoculation induced a controlled oxidative response, possibly linked to improved stress signaling mechanisms.

#### Malondialdehyde (MDA)

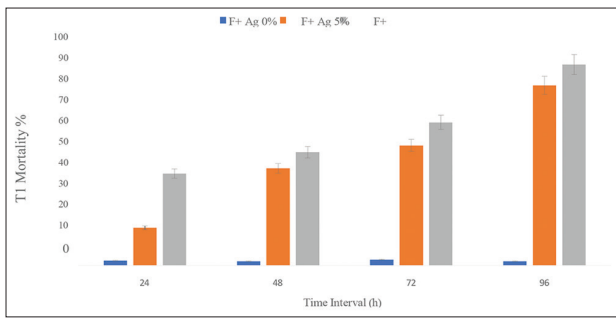
Lipid peroxidation, as measured by malondialdehyde content, was significantly enhanced by nanoparticle and fungal treatments. The maximum MDA value (22.22 mg g<sup>-1</sup> FW) was observed in T2 (10% Ag + 2% F), followed by T5 (2% F). (Figure 3f) depicts that higher concentrations of AgNPs, particularly in combination with fungal inoculation, promoted MDA accumulation,

indicating an increase in oxidative metabolism within chickpea plants.

#### Mortality of *Helicoverpa armigera*

##### T1 mortality (5% and 10% AgNO<sub>3</sub> + 2% F)

The mortality of *H. armigera* larvae fed on chickpea leaves and pods treated with 5% and 10% AgNPs + 2% fungus showed significant increases with both nanoparticle concentration and exposure time. Figure 4 confirms that mortality rates rose consistently as AgNP concentration increased from 5% to 10% in combination with fungal treatment, with longer exposure periods producing higher mortality levels.



**Figure 4:** Showed that T1 mortality increased with concentration of nanoparticles AgNO<sub>3</sub> (5% and 10% with 2% fungus) as well as time interval increased

### T2 mortality (5% and 10% AgNO<sub>3</sub>)

Larval mortality was also significantly influenced by silver nanoparticles alone. Maximum mortality was observed at 10% AgNPs, while lower values were recorded for 5% AgNPs. As shown in Figure 5, mortality increased steadily with higher concentrations of AgNPs, confirming their potent insecticidal effect even without fungal inoculation.

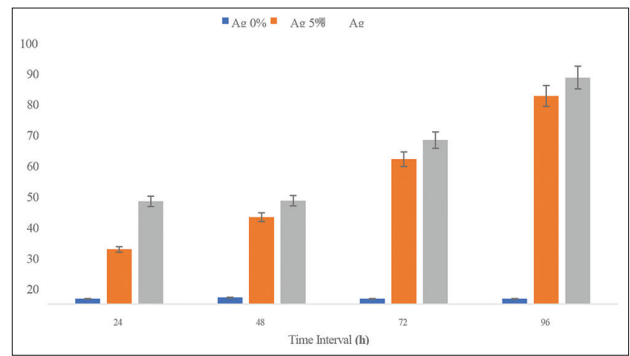
### T3 mortality (2% and 4% Fungus)

When chickpea plants were treated with different concentrations of *M. anisopliae* fungus (2% and 4%), mortality of *H. armigera* larvae increased significantly with fungal concentration and time of exposure. Figure 6 illustrates that mortality was greatest at 4% fungal treatment, demonstrating the effectiveness of *M. anisopliae* as a biological control agent against *H. armigera*.

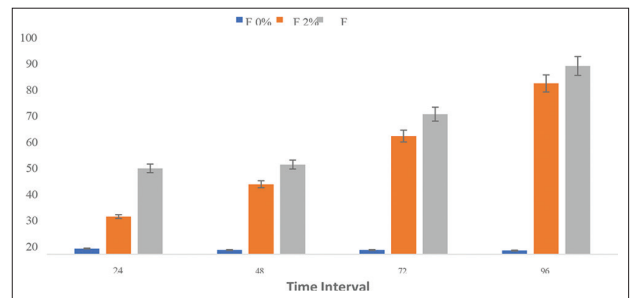
## Discussion

Legumes, particularly chickpeas (*C. arietinum*), are considered one of the most important crops in the Fabaceae family and rank as the third most significant cultivated legume globally. Often called the “meat of the poor,” pulses play a vital role in ensuring food and nutritional security for millions of people worldwide (Kebede, 2020). Due to their high protein content and adaptability to marginal soils, pulses are typically cultivated in resource-poor environments, particularly as secondary crops. However, increasing global population pressures, coupled with decreasing per capita availability of pulses, has heightened the demand–supply gap. As a result, many governments have had to resort to large-scale imports to meet the growing consumption demand (Akibode & Maredia, 2012). Chickpea cultivation is further challenged by abiotic and biotic stresses, among which insect pest damage is one of the most destructive constraints limiting productivity and sustainability.

Among insect pests, *H. armigera* is widely recognized as one of the most serious pests of chickpea, often causing devastating yield losses in addition to reducing grain quality (Ogih *et al.*, 2019). This polyphagous pest, along with aphids, thrips, and caterpillars, attacks different



**Figure 5:** Showed that T2 mortality increased with concentration of nanoparticles AgNO<sub>3</sub> (5% and 10%) as well as time interval increased



**Figure 6:** Showed that T3 mortality increased with concentration of *Metarhizium anisopliae* fungus with (2% and 4%) as well as time interval increased

plant tissues including leaves, pods, and stems, delaying plant development and reducing final yields. Conversely, beneficial insects such as pollinators (e.g., bees) and natural predators (e.g., ladybird beetles) contribute positively to chickpea production by enhancing pollination and suppressing pest populations. Thus, understanding the balance between destructive and beneficial insect interactions is crucial in developing sustainable pest management strategies. Integrated Pest Management (IPM), which combines cultural practices, biocontrol agents, and eco-friendly interventions, has increasingly been recognized as the most sustainable approach in chickpea cultivation systems (Chandrashekar *et al.*, 2014).

Recent advances in nanotechnology have opened new opportunities in pest management. Green-synthesized silver nanoparticles (AgNPs) and entomopathogenic fungi such as *M. anisopliae* have shown great potential as eco-friendly alternatives to chemical pesticides. AgNPs are widely studied for their antimicrobial properties, and their application as foliar sprays or soil amendments has been reported to deter insect pests and pathogens (Ramezani *et al.*, 2019). Similarly, *M. anisopliae* is a well-known entomopathogenic fungus that infects and kills a broad range of insect pests, including *H. armigera*, by penetrating the cuticle and proliferating within the host body (Patel *et al.*, 2014). The synergistic use of AgNPs with entomopathogenic fungi in IPM provides a dual mechanism of pest suppression, whereby nanoparticles damage insect tissues while fungi colonize and kill larvae. Previous findings support this integrative approach, demonstrating reduced pest populations and enhanced

plant vigor under combined treatments (Chandrashekar *et al.*, 2014).

However, despite their effectiveness, AgNPs have also been reported to produce variable physiological effects on plants. Studies have shown both inhibitory and stimulatory impacts, depending on nanoparticle size, concentration, and plant species (Cao *et al.*, 2021). In *Arabidopsis*, AgNPs were observed to increase reactive oxygen species (ROS) production while simultaneously enhancing root development and chloroplast biogenesis, suggesting that nanoparticles can modulate key metabolic pathways related to growth. Antibacterial studies further confirm that AgNPs synthesized from different plant extracts or silver nitrate exhibit strong inhibitory activity against various bacterial strains, though their efficacy depends on the type of plant extract, nanoparticle concentration, and bacterial species involved (Patra & Baek, 2017; Alsammarrarie *et al.*, 2018). The antimicrobial activity of AgNPs is attributed largely to silver ion release, which disrupts microbial cell walls and interferes with metabolic functions (Liu *et al.*, 2012). Additionally, phytochemicals associated with plant-based synthesis may contribute to enhanced antibacterial properties (Abdel-Aziz *et al.*, 2014).

The impact of AgNPs on plant morphology and physiology has been well documented. Comparative studies show significant differences in morphological parameters such as shoot and root length, leaf dimensions, and biomass accumulation between treated and control plants (Jiang *et al.*, 2012; Kaur, 2017). AgNP-treated plants often develop more aerial roots, root branches, and leaves, suggesting a stimulatory effect on growth processes. Furthermore, enzymatic antioxidant activity in plants exposed to AgNPs under biotic stress conditions has been reported to increase significantly. In rice, SOD, POD, and CAT activities were markedly higher in AgNP-treated plants compared with controls, highlighting the role of nanoparticles in enhancing stress tolerance (Duan *et al.*, 2018; Chen *et al.*, 2022). Similarly, photosynthetic pigment analysis has revealed mixed responses, with chlorophyll a showing no significant variation, while chlorophyll b and carotenoid concentrations often increased following nanoparticle treatments (Cantabella *et al.*, 2017; Noori *et al.*, 2020). Although lipid peroxidation levels were largely unchanged in control and AgNO<sub>3</sub>-treated seedlings, AgNP exposure induced significant increases in malondialdehyde (MDA), suggesting oxidative stress (Khan *et al.*, 2019).

Most importantly, the combined application of AgNPs and *M. anisopliae* has demonstrated strong insecticidal activity against *H. armigera*. Laboratory and field studies consistently report significant larval mortality at higher concentrations of nanoparticles and fungal spores, with mortality rates ranging from 60% to over 90% depending on exposure time and dosage (Allahyari *et al.*, 2020; Venkata *et al.*, 2022). The larvicidal properties of AgNPs derived from various plant extracts, including *Azadirachta indica*, *Ficus religiosa*, and *Ocimum* spp., confirm their potential as effective biopesticides (Qamar *et al.*, 2021; Chinnasamy *et al.*, 2023). Moreover, nanoparticle treatments have been

shown to delay larval development, reduce detoxifying enzyme activity, and decrease body weight, thereby further suppressing pest populations (Anees *et al.*, 2022). These findings are in agreement with previous studies highlighting the pathogenic potential of *M. anisopliae* against *H. armigera* larvae, where geographical variations in fungal isolates may influence virulence but consistently demonstrate significant mortality (Wakil *et al.*, 2014; Jarrahi & Safavi, 2016; Poopedi *et al.*, 2021; Idrees *et al.*, 2022). Collectively, these results reinforce the importance of integrating green nanotechnology with entomopathogenic fungi as part of sustainable pest management strategies in chickpea cultivation.

## Conclusion

The present study highlights the potential of integrating silver nanoparticles (AgNPs) and *Metarhizium anisopliae* as sustainable and effective strategies for managing *Helicoverpa armigera*, a major pest of chickpea (*Cicer arietinum*). Chickpea, valued for its nutritional, agronomic, and economic importance, suffers significant yield losses due to pest infestations, necessitating eco-friendly alternatives to chemical pesticides. The application of biosynthesized AgNPs, particularly those prepared using *Ficus* leaf extract, in combination with entomopathogenic fungi demonstrated not only enhanced insecticidal activity against *H. armigera* but also beneficial effects on plant physiology. Treated plants exhibited improvements in biomass, root and shoot growth, and photosynthetic pigments, while biochemical analyses revealed elevated levels of reactive oxygen species (ROS), malondialdehyde (MDA), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), alongside increased activities of enzymatic (SOD, POD, CAT) and non-enzymatic antioxidants (TSP). These physiological and biochemical modulations suggest that AgNPs and *M. anisopliae* not only suppress pest populations effectively but also stimulate plant defense mechanisms and growth responses. Furthermore, the mortality rates of *H. armigera* larvae increased significantly with higher concentrations of nanoparticles and fungi over progressive time intervals, confirming the strong larvicidal and synergistic potential of this combined approach. Collectively, these findings underscore that the integration of green nanotechnology and biological control agents can provide an environmentally sound, efficient, and sustainable pest management strategy, reducing reliance on hazardous chemical pesticides while promoting chickpea productivity and resilience.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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