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Phytofabrication and characterization of ZnO nanoparticles with *Anagallis arvensis* as promising eco-friendly insecticide against *Tribolium castaneum* Herbst

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ABSTRACT

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is a significant and economically detrimental pest that infests stored grains, particularly wheat, which serves as a staple food for a substantial portion of the Asian population. Environmental and health hazards of available chemical control options coupled with development of resistance against them warrants discovery of more sustainable and environment friendly pest control materials. This study aimed to investigate the physical properties and insecticidal efficacy of zinc oxide nanoparticles (ZnO NPs), fabricated with *Anagallis arvensis* extracts, against *T. castaneum*. The synthesized ZnO NPs were characterized using various analytical techniques. The UV–Visible spectrograph exhibited a characteristic peak at 320 nm at room temperature, while Fourier Transform Infrared Spectroscopy (FTIR) was employed to determine the organic constituents in NPs. Following that, the Scanning Electron Microscopy - Energy Dispersive X-ray (SEM-EDX) analyses were conducted to assess the shape, size, and weight of the nanoparticles. Dynamic Light Scattering (DLS) analysis provided information on their surface charge and hydrodynamic diameter. The mortality bioassay demonstrated that ZnO NPs exhibited a significant toxic effect against *T. castaneum* ($p < 0.05$), with mortality showing a direct correlation with the concentration and exposure time. The highest dose resulted in a mortality rate of 75 % at the end of the experiment, and the LC_{50} calculated through Probit-mortality analysis was 105.47 mg/L. Our findings suggest that ZnO NPs loaded with plant extracts hold exciting potential as effective grain protectants.

1. Introduction

Nanotechnology is an exciting field of modern world that deals with nano-array materials of 1–100 nm size, and have become a solution for many problems. Among many nanoparticle synthesis methods, the green synthesis approach, which uses fungi, bacteria, and plants to synthesize nanoparticles, is relatively more eco-friendly and an efficient method. In green synthesis, smaller bioactive substances such alkaloids, terpenoids, polyphenols, and flavonoids actively participate in the synthesis of nanoparticles (Bachheti and Bachheti, 2023). Working on the similar lines, the green fabrication of metallic nanoparticles with a potent plant extract can be even more cost-efficient and environment-friendly approach (Parveen et al., 2016). This way the phytofabricated nanoparticles carry the potential of saving world from hunger by targeting pests in stored grains including wheat (Affrald, 2024).

Being a primary source of carbohydrates dietary fibers and proteins and other vital nutrients (Seal and Brownlee, 2015), cereal grains are consumed up to 2.81 billion tons as staple food in many parts of the world (Thielecke and Nugent, 2018). Among all cereals, wheat, rice and maize provides approximately 60 % of the world's total energy intake by food (Kahn, 2021) where wheat alone fulfils dietary requirement of at least 40 % of the total population (Thiagarajan and Ramasubbu, 2021). Currently, Pakistan is ranked 8th in wheat producing countries where 75 % of total production is contributed by Punjab province (Khan et al.,

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2021). With anticipated global population to be doubled by 2050 pose a serious challenge to food security (Dissanayaka et al., 2020, Shendekar et al., 2023). The grains are stored for prolonged periods, and become vulnerable to pest attacks, and bear significant losses especially due to insect pest infestations (Pires et al., 2017). According to an estimate, at least 5 % of grains are lost during the storage period due to various factors before going to their final consumers (Padın et al., 2002) and these losses are not only due to insect feeding, but also the nutritional quality is deteriorated as a result of insect and fungal infections (Zuhra et al., 2018). Among major insect pest species, red flour beetle (*Tribolium castaneum* Herbst) is found infesting the grain storages throughout the world (Abdullahi et al., 2019). Currently the grain industry rely on fumigants and structural insecticides, but its frequent use not only resulted into insecticidal resistance, but also have serious environmental and health hazards (Kim et al., 2010), Likewise *T. castaneum* is also reported to have developed strong insecticide resistance against a phosphine (Huang et al., 2019) and other structural insecticides including bifenthrin (Julio et al., 2017), carbaryl (Gao et al., 2018), cyhalothrin, cypermethrin, deltamethrin (Collins, 1990, Liang et al., 2015), diflubenzuron (Rösner and Merzendorfer, 2020), malathion (Haubruge et al., 2002) and permethrin (Pyrethroids) (Oppert et al., 2015). This warrants the discovery of novel compounds for stored grain pest management that are more efficient, sustainable, and environmentally safe to use. Plant based extracts are less soluble in water and are adsorbed on grain surfaces making them relatively less efficient and more persistent in the environment (Stejskal et al., 2021). Nanotechnology provides viable solutions to overcome such problems by enhancing water solubility and improving the dissolution rate of such nanoparticles for enhanced dispersion uniformity when applied (Margulis-Goshen and Magdassi, 2013).

Anagallis arvensis L is known for its ethnopharmacological importance (Saleem et al., 2021) and antimicrobial, antifungal, anti-inflammatory and antiviral properties (Yasmeen et al., 2020, Sharf et al., 2021). In this study we have used eco-friendly approach for the green synthesis of ZnO nanoparticles. To our knowledge this is a first study of ZnO NPs preparation from *Anagallis arvensis* L as reducing and capping agent for development of pesticide against *T. castaneum*.

2. Materials & methods

2.1. Insect culturing

One week old adults of *T. castaneum* were retrieved from the laboratory cultures maintained at the laboratory at COMSATS University Islamabad (CUI), Islamabad, Pakistan. These beetles were introduced in freshly milled whole meal wheat flour (200 g) kept in plastic containers (250 ml) containing and incubated at 25 ± 2 °C and 65 ± 5 % r.h. in 8:16 h light and dark regime. The flour was pre-enriched with 5 % baker's yeast (w/w) to enhance oviposition (Đukić et al., 2021). After 10 days of incubation, adults were removed from the jars and cultures were observed frequently until the 3rd instar larvae were developed for further bioassays.

2.2. Plant extract preparation

Whole plants of *A. arvensis L.* were collected from a wheat field near *Kallar Syedan* region in Rawalpindi district of the Punjab province in Pakistan (33° 24′ 52″ N and 73° 22′ 43″ E). After taxonomic confirmation of the plant species, the samples were washed under tap water followed by rinsing with double distilled water thrice to clean any dust and impurities. For extract preparation, the methods described by Fakhari et al. (2019) were followed. The whole plant was shade dried for 10 days and then powdered in an electric coffee grinder. Aqueous solution was prepared by stirring 8 g of powdered plant material in 250 ml of distilled water and heated at 85 °C for 30 min on a hot plate magnetic stirrer. The suspension was cooled and filtered through Whatman filter paper No. 1and was kept at room temperature for future use.

2.3. Phytofabrication of ZnO NP with A. arvensis extracts

Zinc oxide (ZnO) nanoparticles (NP) were fabricated by co-precipitation method described by Fakhri et al. (2019). One molar solution of Zinc nitrate (Sigma-Aldrich & Merk KGaA, Darmstadt, Germany) was prepared in 50 ml of double distilled water. To determine the best optimal concentration of Zinc oxide nanoparticles, we proceeded with variable volumes of plant extract (15 ml, 20 ml, 25 ml, 30 ml and 35 ml) in a glass flask, and at 30 ml NPs concentration was optimized, for bulk preparation 30 ml of plant extract was taken and mixed with 50 ml of zinc nitrate solution, then for 2 h, heat the mixture on magnetic plate (AREC, VELP SCIENTIFICA, Europe) at 80 °C and 800 rpm till it turned deep brown in color. The solution was allowed to cool at room temperature and then centrifuged at 7000 rpm at room temperature for 5 min with Allegra X-30R Centrifuge (BECKMAN COULTER, Germany) to get pellets of ZnO NPs. The pellets, were retrieved, and washed repeatedly with double distilled water and oven-dried at 60 °C for 5H and crushed to get fine NPs using pestle mortar. The prepared nanoparticles were then sonicated at 70 °C for 30 min in a sonication chamber (Elmasonic E-30H, Germany) and the prepared stock solution were stored for further bioassays.

2.4. Characterization of zinc oxide nanoparticles

Since physical and chemical properties of nanomaterials are significantly associated with their biological potentials and stability, we conducted a detailed characterization of our prepared ZnO green nanoparticles using various techniques. Synthesized ZnO NPs were structurally characterized by UV–Visible Spectrophotometer (SPECORD 200 plus Analytikjena, Germany) within I200-800 nm wavelength range. Fourier Transform Infrared Spectroscopy (FTIR) is a popular analytical technique to determine organic constituents which is present in a sample by finding their specific rotation and vibrational modes. The synthesized ZnO nanoparticles were analyzed using FTIR (FT/IR-4100 Spectrometer, JASCO, Japan) within the wavenumber range of 600–4000 cm^{-1} (mid-IR) by the KBr method. The appearance and size of particles were observed using Scanning Electron Microscopy (SEM) (Tescan MAIA3, Europe) while the elemental details and composition of nanomaterials were determined using an Energy-Dispersive X-ray (EDX) Spectrometer (Malvern Panalytical Ltd, UK). Dynamic light scattering (DLS) was performed using Zetasizer Nano Series (Malvern Panalytical Ltd, UK) to determine the stability and particle size of ZnO NPs. Homogeneity in size distribution and mean particle size of nanoparticles were recorded and represented as Zeta potential (ζ) and hydrodynamic diameters to demonstrate NP's surface charge and effective formulation preparations.

2.5. Mortality bioassay

The insecticidal efficacy of prepared nanoparticles was assessed against 3rd instar larvae of *T. castaneum* under laboratory conditions. Five concentrations of phytofabricated ZnO NP, i.e., 50 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, 500 mg/L, 1000 mg/L and 2000 mg/L were prepared in double distilled water to be used in the bioassays. The experiment was replicated five times. For each treatment, 10 g of wholemeal wheat flour was treated with 3 ml of prepared solutions and for a control treatment, 10 g of whole wheat was treated with 3 ml of distilled water by using a micro sprayer and was left at room temperature for drying. From each treatment, 2 g of treated flour was transferred from all treatments and controls to Petri dish and 10 larvae (3rd instar) were released in each Petri dish. The data for larval mortality were recorded 24, 48, 72 h after application (HAA) then it was examined on the 6th, 8th, 10th, 13th' and 15th day after application (DAA). The larvae were deemed dead if found unresponsive to a probe, dried or discolored.

2.6. Statistical analyses

The data regarding larval mortality was corrected (Abbott, 1925) and checked for homoscedasticity (Levene, 1960) prior subjecting to one-way analysis of variance (ANOVA) using SPSS data analysis software. Where significant differences among different concentrations were recorded, the means were separated through post-*hoc* pairwise comparisons using Tukey's HSD test ($p < 0.05$). The LC_{50} value was estimated by performing probit analysis using LeOra Software Polo-PC.

3. Results

3.1. Characterization of ZnO nanoparticles

UV–visible spectroscopic analysis was done to check the absorbance of prepared NPs and understanding their chemical and physical properties. I revealed that zinc ions present in the solution were reduced to zinc oxide by metabolites present in the extract of *A. arvensis*. Metabolites in the extract not only reduced the zinc ions but also stabilized them. It was ensured by UV–visible spectrum with a ZnO nanoparticle's characteristic peak at 320 nm at room temperature (Jayachandran et al., 2021) in nano-solution (Fig. 1A). The absorption peak in UV–visible spectroscopy for ZnO nanoparticles was recorded within the wavelength range of 310 – 360 nm (Ghamsari et al., 2017). Bandgap energy was calculated by using the formula Eg (eV) = 1240/ λ, where λ is the absorption intensity which arises from the baseline of the spectrum. The baseline absorption intensity was recorded as 320 nm, as a result, the bandgap energy of ZnO NPs had been determined to be 3.87 eV, which is comparable to the values reported by Tan et al., (2005).

FTIR was performed to determine the changes in functional groups.

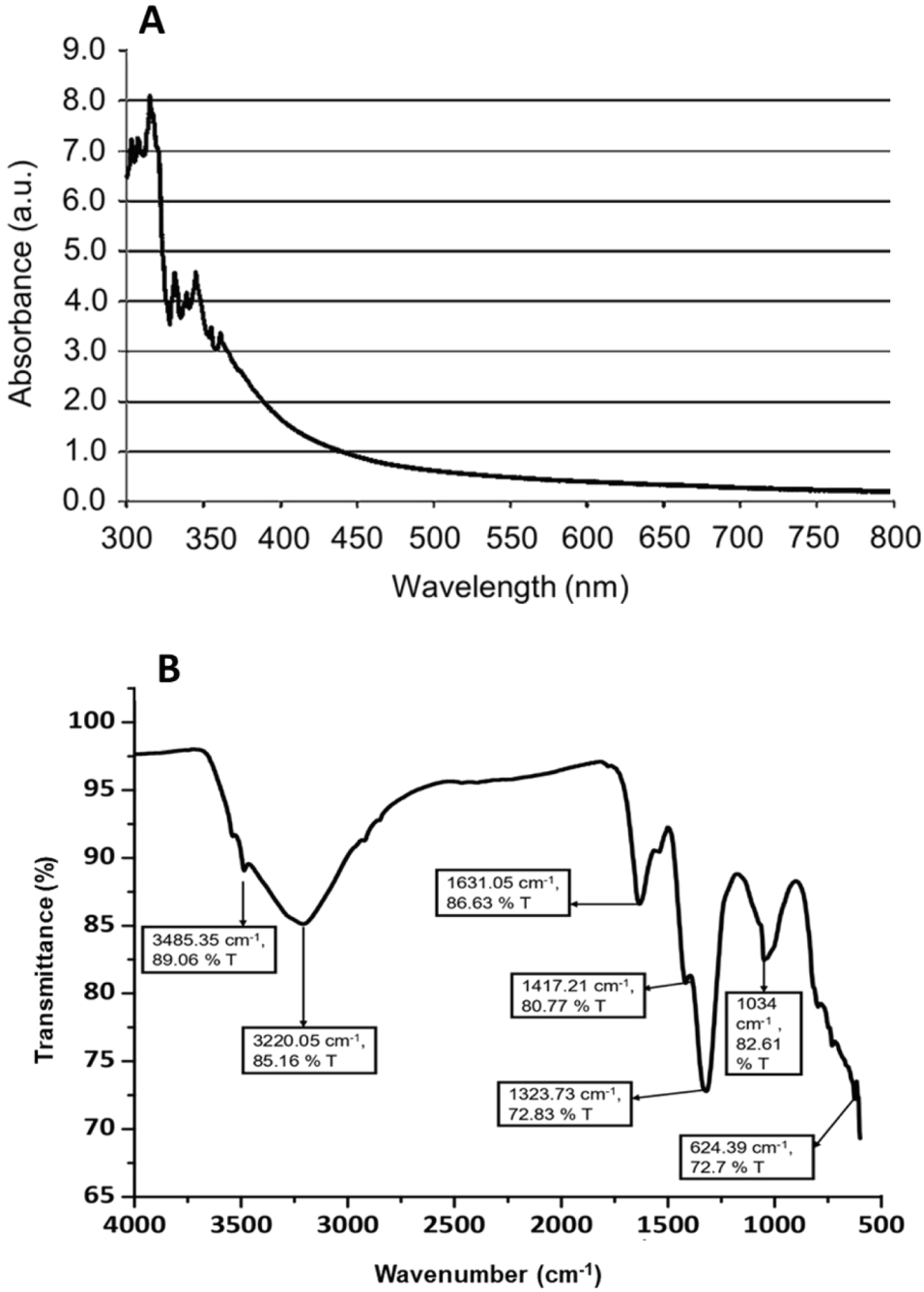

Fig. 1. UV–visible spectra (A) and Fourier Transform Infrared Spectra (FTIR) (B) of ZnO nanoparticles fabricated with *Anagallis arvensis* extract.

FTIR spectrum of ZnO nanoparticles exhibited absorption bands at 3485.35 cm^{-1}, 3220.05 cm^{-1}, 1631.05 cm^{-1}, 1417.21 cm^{-1}, 1323.73 cm^{-1}, 1034 cm^{-1} and 624.39 cm^{-1} for functional groups (Fig. 1B). The absorption peaks were seen in single bond, double bond, and fingerprint region, while there was no considerable peak present in the triple bond region. Broad stretch at 3485.35 cm^{-1} and 3220.80 cm^{-1} indicated the presence of the hydroxyl (O-H) group. Stretching at 1631.05 cm^{-1} showed the presence of alkene or carboxyl/amide group. Bending at 1417.21 cm^{-1} was due to the Vinyl C-H in-plane bend. A sharp peak at 1323.73 cm^{-1} shows the presence of an aromatic amino group while absorption band at 1034.43 cm^{-1} was due to in-plane C-H bending and the vibrational band arise at 624 cm^{-1} due to O-H bending. It is reported in the literature that absorption bands found below 1000 cm^{-1}, which is known as the fingerprint region, are due to metallic oxides (Kaur et al., 2019, Din et al., 2021, Harikrishnan et al., 2022). The prepared ZnO nanoparticles were found to be stabilized, reduced, and capped due to loading of *A. arvensis* plant extracts.

ZnO NPs in this study were characterized by the Dynamic Light Scattering (DLS) method after proper sonication. The measurement of ZnO nanoparticles in liquid form was taken at pH 6.5, and surface charge of NPs in solution is measured as zeta potential. Zeta potential refers the surface charge exhibited by nanoparticles when dispersed in a solution. This parameter serves as a crucial tool for comprehending the condition of the nanoparticle surface and forecasting the enduring stability of a colloidal dispersion. The zeta potential value (-24.8 mV) (Fig. 2A) with higher negative and greater hydrodynamic value (169.2 nm) (Fig. 2B) affirms the strong repulsion between ZnO nanoparticles and so that increases its stability. It also indicates that synthesized material has large electrostatic forces and will ultimately make a stable suspension formulation for use in pest management.

The SEM analyses was done to study the topology of NPs. It was demonstrated that microstructure exhibited a compact particle structure of ZnO NPs, with irregular shape and nanoclusters were observed (Fig. 3A and B). Energy Dispersive X-ray Spectrometric (EDX) analysis revealed that the sample contains a significant amount of zinc as well as oxygen in the form of zinc oxides (Fig. 4). Weight percentage peaks of these are comparable to previous literature for confirmation of ZnO nanoparticles (Jayachandran et al., 2021). Apart from oxygen and zinc, a trace amount of phosphorous (4.25 % at 2.0 eV) was also witnessed in the EDX spectrum. The presence of phosphorus shown in the spectrum (Fig. 4) specifies the biomolecule involved in capping and reducing ZnO nanoparticles.

3.2. Mortality bioassays

The data after 24 h of application of ZnO nanoparticles loaded with *A. arvensis* plant extract demonstrated a significant difference among all concentrations evaluated against *T. castaneum* ($F_{8,\ 44} = 4.12$; $p = 0.01$).

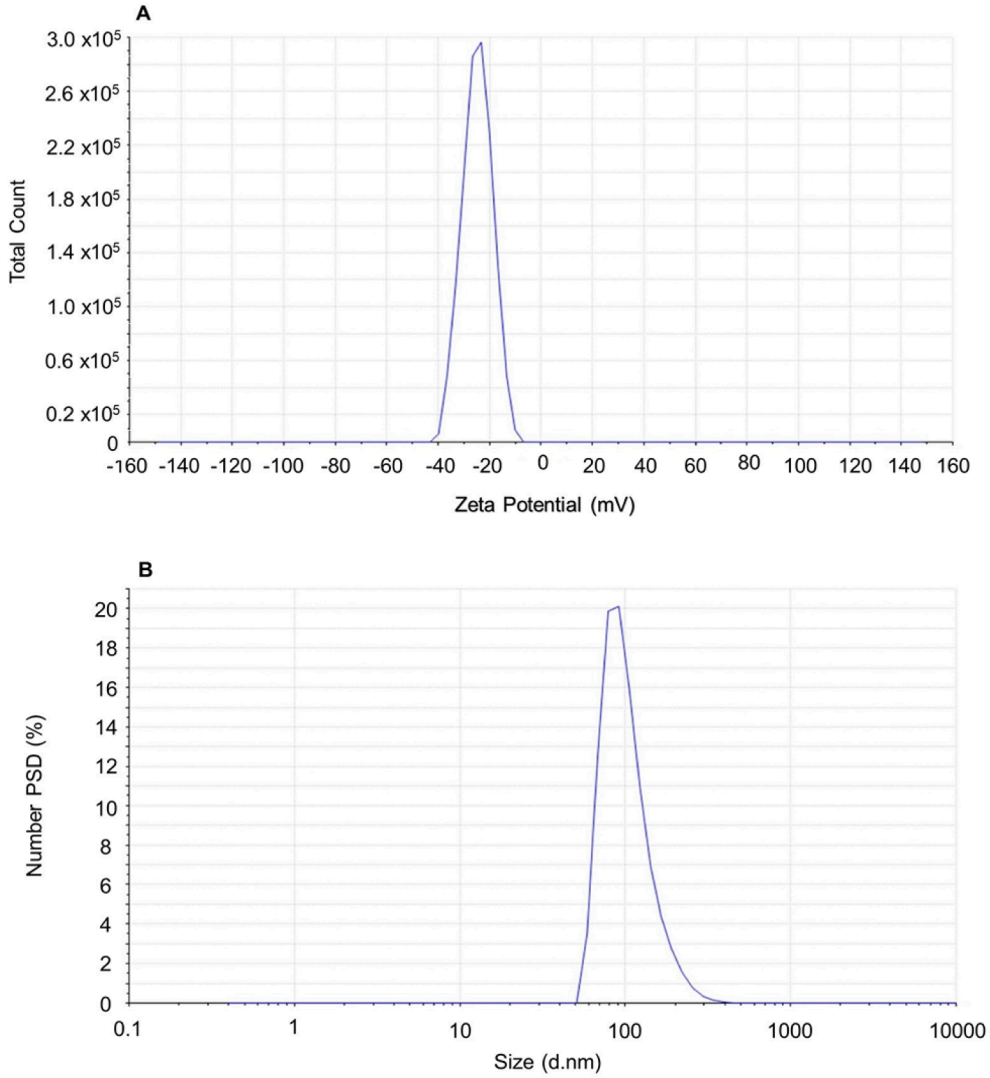

Fig. 2. Zeta Potential (A) and Z-average Size (hydrodynamic diameter) (B) of ZnO nanoparticles fabricated with *Anagallis arvensis* extract.

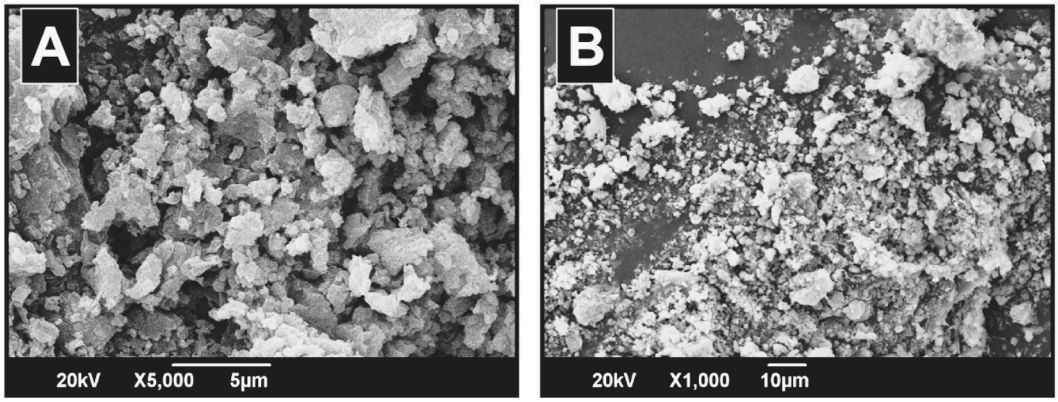

Fig. 3. Scanning Electron Microscopy (SEM) images of ZnO nanoparticles.

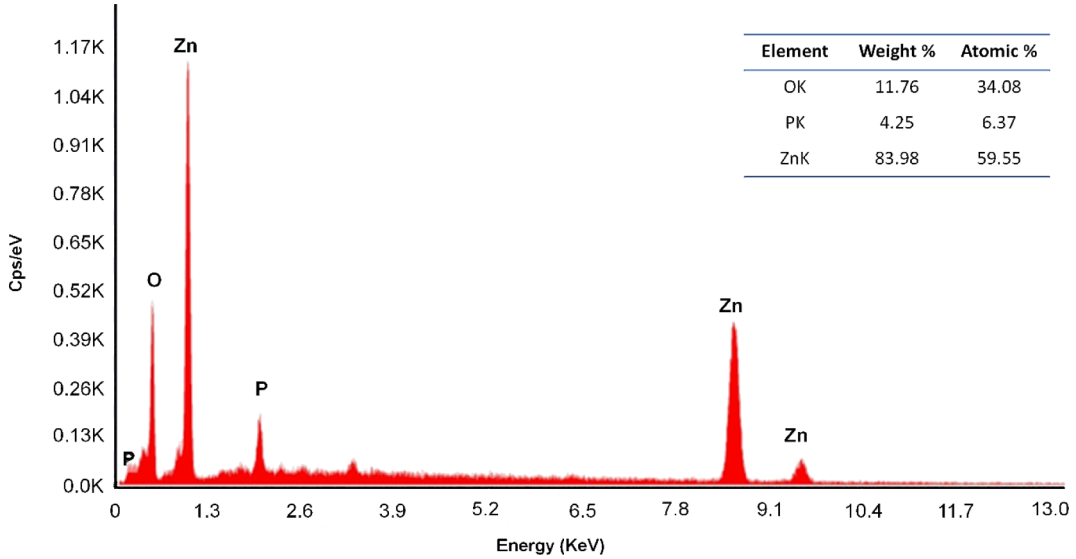

Fig. 4. Energy Dispersive X-ray spectrometric (EDX) analysis of ZnO nanoparticles performed by Field Emission Scanning Electron Microscopy (FESEM-EDX).

The highest mortality of *T. castaneum* larvae had been observed when they were treated with 1000 mg/L concentration (Fig. 5A), whereas the control larvae did not show any significant mortalities and it had no significant difference as compared to that when 100 mg/L and 200 mg/L doses were given to larvae (Fig. 5A). All other concentrations i.e., 300–500 mg/L had significantly higher mortalities as compared to the control. Surprisingly, 2000 mg/L was less effective than 1000 mg/L concentration on the first day after application.

After 48 h of application different concentrations of treatment showed significant differences in mortality against larvae of red flour beetles ($F_{8,\ 44\ =}$ 2.89; $p = 0.014$). On the second day of application of green fabricated ZnO nanoparticles, 1000 mg/L concentration was recorded with the highest mortality of *T. castaneum* larvae followed by 400 mg/L and 500 mg/L concentrations (Fig. 5B). Interestingly, after 48 h 2000 mg/L concentration remained less significant than 1000 mg/L concentration. While control had fewer mortalities than all other concentrations, the difference between control and 50–300 mg/L concentrations were non-significant as compared to higher doses of treatment (Fig. 5B).

Results assured that after 72 h of application, significant mortalities of *T. castaneum* larvae were noticed in all concentrations of ZnO NPs ($F_{8,\ 44\ =}$ 2.938; $p = 0.012$). The significant highest mortality was observed in 400 mg/L concentration than in 1000 mg/L followed by 500 mg/L concentration (Fig. 5C). Control had no such mortalities on the 3rd day after application and every other concentration had a significant difference to control. Concentration 50—300 mg/L were not shown higher differences in mortality than in control concentration (Fig. 5C).

Results concluded a significant difference ($F_{8,\ 44\ =}$ 3.157; $p = 0.008$) between all concentrations on the 6th day after application of nanoparticles. The highest mortality was observed in treatment with 500 mg/L concentration on the 6th day which is more than 50 than in 1000 mg/L and 400 mg/L (Fig. 5D), whereas mortalities in control was not much significant. Nanoparticles concentrations 50–300 mg/L and 2000 mg/L has less mortalities of larvae as compared to 400 mg/L and 1000 mg/L concentrations (Fig. 5D).

In the treatment of metallic nanoparticles of zinc oxide loaded with plant extract of *A. arvensis*, data showed a significant difference in larval mortality in all concentrations ($F_{8,\ 44\ =}$ 6.674; $p < 0.001$). Concentration 1000 mg/L had significantly highest mortality followed by 2000 mg/L concentration (Fig. 5E). All other concentrations of nanoparticles had a considerably higher difference in mortalities than mortalities in control (Fig. 5E).

The results of the variance test on the 15th day represent a constant increase in mortalities with time from lower to higher concentration (Fig. 5F). Graph demonstrated that all concentrations of ZnO NPs synthesized with *A. arvensis* extract had a significant difference in mortality ($F_{8,\ 44\ =}$ 18.232; $p < 0.001$). An abrupt increase in mortalities was seen till the first ten days after the application of treatment then it became gradual after the 11th day with treatment concentration (Fig. 5F). Significant higher mortality was observed in 2000 mg/L concentration on

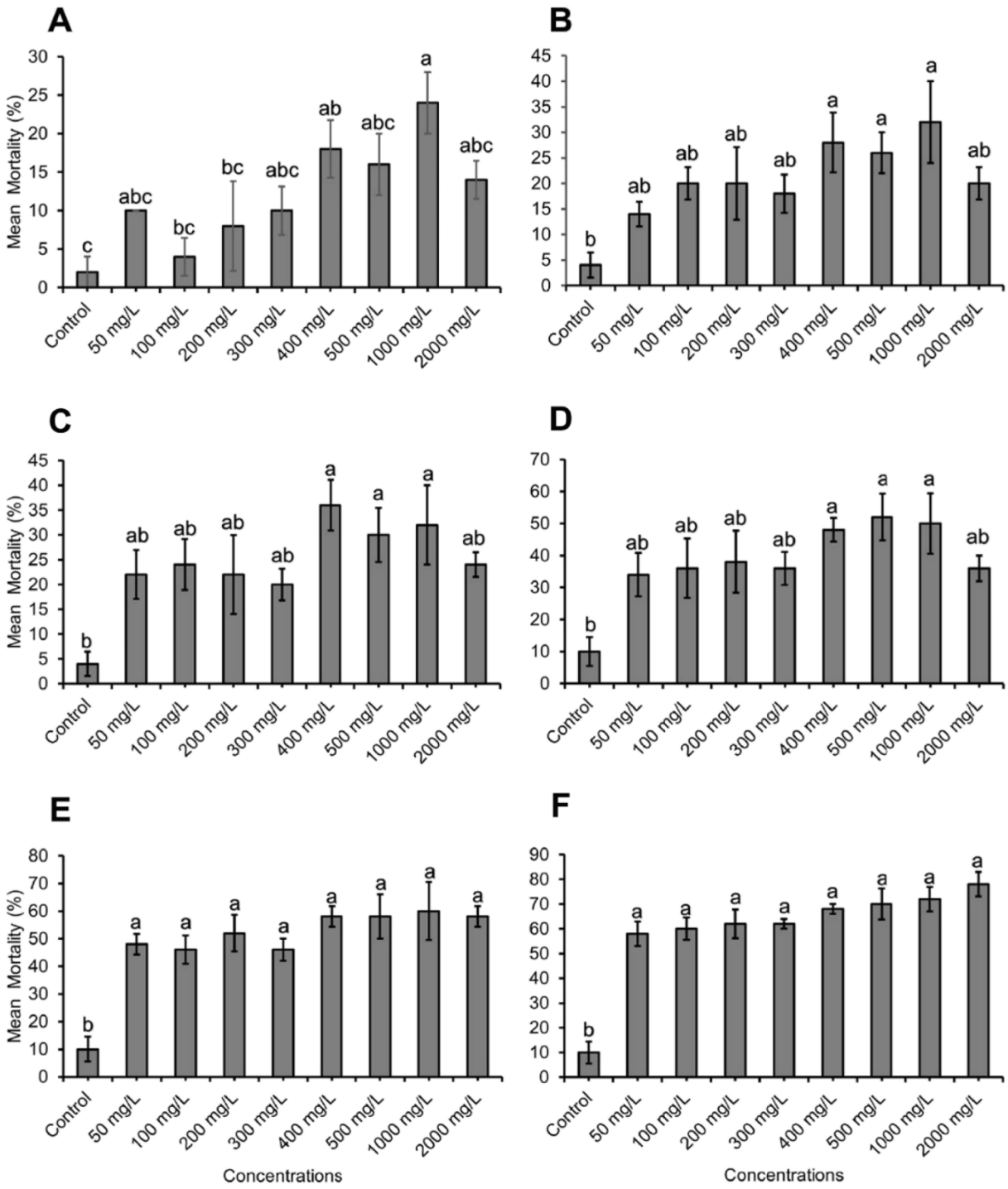

Fig. 5. Comparative mortality of *Tribolium castaneum* when treated with different concentrations of ZnO nanoparticles fabricated with *Anagallis arvensis* extracts at 24 h (A), 48 h (B), 72 h (C), 6th day (D), 10th (E) and 15th day (F) after applications. The bars present mean mortalities (%) ± SEM and the superscript letters atop giving the post-*hoc* pairwise comparison between treatments. The bars with different letters are statistically different from one another (Tukey's HSD; $p < 0.05$).

the 15th day while the control has a very a smaller number of dead larvae on the same day (Fig. 5F). In general, it was observed that the mortality increased with the increase in exposure times (Fig. 6). All the concentrations resulted into significantly higher mortalities as compared to the control and concentrations above 400 mg/L had demonstrated higher efficacies (Fig. 6).

The data was subjected to probit analyses to determine the lethal concentration of ZnO NPs against *T. castaneum* (Fig. 7). The LC_{50} was calculated as 105.47 mg/L with fiducial limits of 15.09–736.82 ($\chi^2 = 1.000$; $R^2 = 0.916$).

4. Discussions

Green synthesized metallic nanoparticles not only have a noticeable potential to be used as insecticide against *T. castaneum* larvae (Jafer and Annon, 2018), but also have a synergistic effect when mixed with malathion to treat malathion resistant populations (AS and Thangapandiyan 2019). Even biologically fabricated *Pseudomonas fluorescens* strain based copper nanoparticles significantly inhibit *T. castaneum* population development and survival at very low concentrations (El-Saadony et al., 2020). We have observed a similar impact of application of green

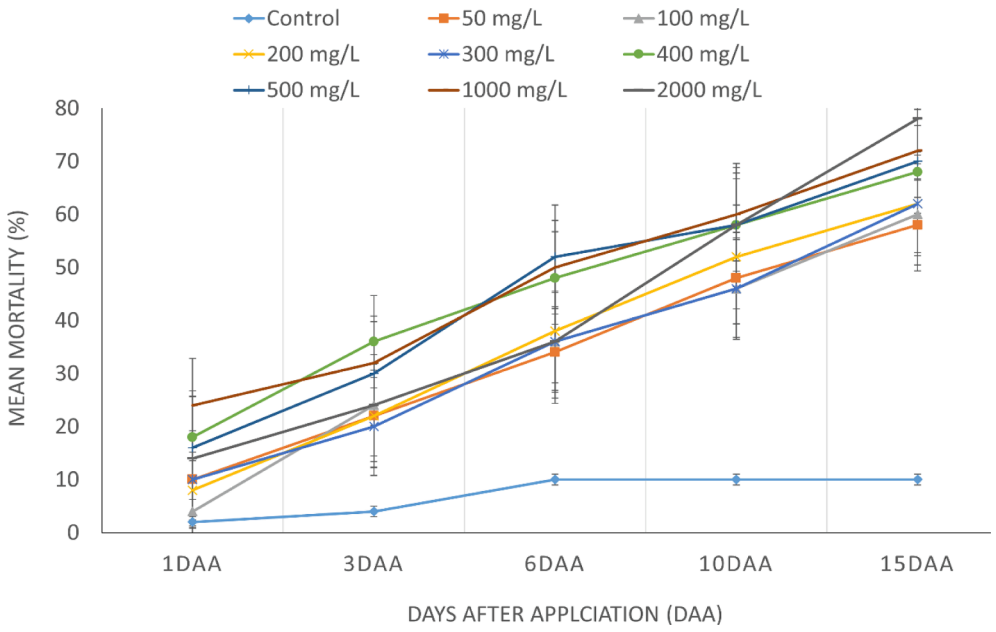

Fig. 6. Effect of exposure time on mortality due to exposure of different concentrations of ZnO nanoparticles fabricated with *Anagallis arvensis* extracts.

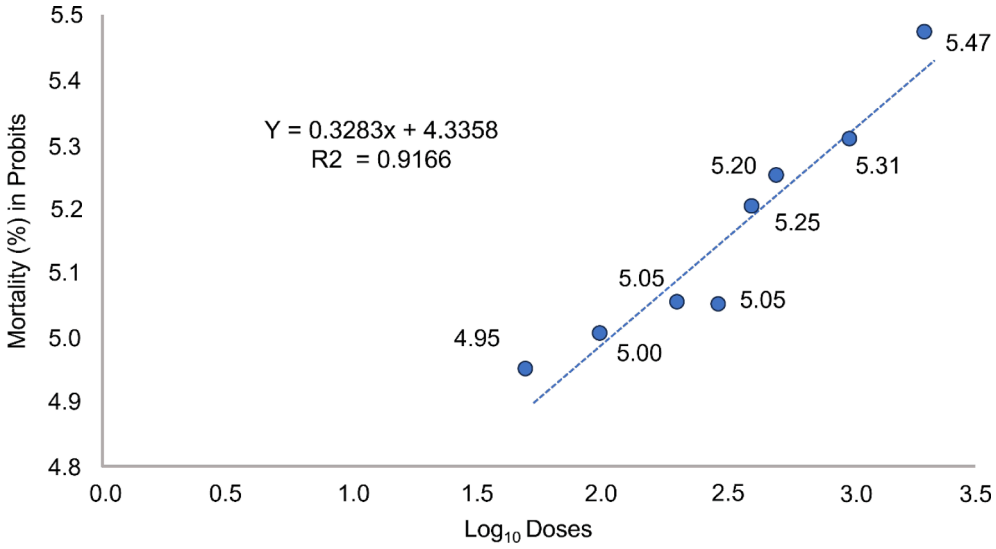

Fig. 7. Log concentration-mortality regression line for the activity of ZnO nanoparticles fabricated with the extract of *Anagallis arvensis* against *Tribolium castaneum*.

synthesized ZnO nanoparticles loaded with *A. arvensis* plant extracts. In general, mortality of *T. castaneum* larvae increased with increasing the concentration of ZnO nanoparticles where about 75 % of the larvae were killed when treated with 2000 mg/L concentration of ZnO nanoparticles. Not only the increase in concentration, but also the exposure time had a direct impact on larval mortality. The mortality increased from the first day after application and the highest mortality was observed on day 15 after application. These results perfectly align with the findings already reported (Lakshmi et al., 2020, Hilal et al., 2021). Along the experiment, we observed that the mortality was less associated with concentration in the initial 10 days of exposure, however, after that a clear direct association was observed, as described earlier. We assume this may be due to the selective feeding by the larvae in the initial days, while in the later days they did not have much of the choice left, hence on day 13 and 15 after application the larvicidal effect increases uniformly from lower to the higher dose. Our results demonstrated that minimum larval mortality was observed by the first two days after exposure. At the end of the experiment, more than 50 % mortality was observed in all doses. Variance results clarified that there was a significant difference in control and all other doses of metallic nanoparticles.

Zeta potential is a physicochemical terminology used to determine the surface charge of particles in bio colloidal systems. It gives information about the stability of nanoparticles and helps in determining their state. Nanoparticles have their specific charge which attracts the thin layer of opposing charge ions towards their surface; zeta potential is the dually layered electric potential that generates at the bio-colloids boundary. Dynamic light scattering analysis revealed that ZnO NPs used in this study have good stability and it also has a smaller hydrodynamic diameter which indicates better penetration of green synthesized ZnO NPs. Our particles have the zeta potential value of −24.8 mV, this negative charge value of zeta potential is because of reducing agents like phenolic compounds or flavonoids of plant extract that is used in this study to reduce the Zinc Oxide ions into ZnO nanoparticles, zeta potential value of our NPs is comparable with maximum stabilization value of nanosuspension which is −30 mV.

Nanoparticles can form aggregates due to electrostatic and Van der walls forces but zeta potential of −24 mV indicates enough aggregation stability of our NPs for its application as insecticide. Results of scanning electron microscopy also revealed aggregates of ZnO NPs. Aggregates of NPs can be dissolved again with solvent by proper sonication because they are not permanently attached to one another. Agglomeration is a term that is more concerning because agglomerated particles are become difficult to disperse as compared to aggregated ones. Since aggregation may influence the release of active ingredients from nanoparticles, controlled release of insecticidal agents is desirable for long-lasting efficacy. In our case we may expect that ZnO NPs prepared may have altered release kinetics, affecting the sustained release of the active compounds to target sites. Scanning electron microscopic analysis demonstrated that green synthesized ZnO nanoparticles do not have any other phases and only ZnO nanoparticles dominates in microscopic image. Particle size, shape, coated material and mechanism of action of nanoparticles have an impact on the effectiveness of nanoparticles (Jiang et al., 2015).

Factors like dose concentration, time and uniform distribution of nanoparticles affect the results of feeding bioassay. It is very obvious that nanoparticles are highly effective when applied at higher concentration and it is less effective when applied for a shorter time span. To get a more efficient result it should be applied for a minimum of two weeks to get more than 75 % mortality. It is assumed that ZnO nanoparticles metabolically affects *T. castaneum* because in treatments actual size of larvae is reduced and destined to death. Mortality ratio is very less in starting days after application of bioassay but at the end of experiment higher mortality percentages are recorded. This factor revealed that nanoparticles have good persistence and their effect increased with time. It has been reported that Al_2O_3 and ZnO nanoparticles when applied on *T. castaneum* inhibits the progeny number and weight loss % effectively as compared to Malathion (Salem et al., 2015).

Metal ions, including zinc, exhibit antimicrobial activity by inhibiting enzymes, generating reactive oxygen species, damaging cell membranes, and hindering microelement uptake by microbes (Li et al., 2010). Zinc oxide nanoparticles, derived from the essential trace element zinc, are of interest for their controlled applications. While zinc is crucial for human health (Maret, 2011), excess free zinc ions can be cytotoxic, particularly to nerve cells (Sindhu et al., 2022). To address this, zinc cations are complexed with bioactive ligands, like proteins, in the synthesis of zinc oxide nanoparticles. This controlled approach ensures the beneficial properties of zinc while minimizing potential negative effects, establishing these nanoparticles as a safe option for various applications (Gudkov et al., 2021).

ZnO in the form of nanoparticles have a very small size which makes Zn absorb easily by the body. Food and Drug Administration of the United State categorized ZnO as a safe substance (GRAS) that's why ZnO Nanoparticles gains much attention in the biomedical field (Jiang et al., 2018).

ZnO is easily soluble in water (Dimapilis et al., 2018) and Microcrystals of ZnO have a wide bandgap and efficiently absorb UV (A, B) regions of light spectra (Mishra et al., 2017). Currently, the synthesis of ZnO nanoparticles has attracted much interest in future applications due to the high excitation binding energy of 60 meV, broad bandgap (3.36 eV) and higher luminescent efficiency of ZnO (Król et al., 2017). Antimicrobial action of ZnO NPs is also well known against many microorganisms include *Bacillus subtilis, Sarcina lutea, Pseudomonas aeruginosa, Pseudomonas vulgaris, Aspergillus niger, Bacillus megaterium, Klebsiella pneumonia, Staphylococcus aureus, Candida albicans and Escherichia coli* (Siddiqi et al., 2018).

Different biologically unsafe techniques which used hazardous and toxic chemicals and high energy input for the preparation of Zn nanoparticles have been developed like spray pyrolysis, chemical vapor deposition, sol–gel, microwave-assisted techniques, precipitation methods, hydrothermal and microwave-assisted techniques (Fakhari et al., 2019). In contrast, the synthesis of nanoparticles through green technology removes the need for high-priced instruments, toxic chemicals and high temperatures (Rajeshkumar et al., 2018). A recent study by Sun et al., (2023) concluded that ZnO NPs did not aggregate nor disturb other nutrient concentrations within wheat grain that's why they are safe to apply by considering food safety measures. Findings of this study indicated that green synthesized metallic nanoparticles evaluated have significantly higher potential to control an infestation of *T. castaneum* in the wheat flour. Similar observations were reported by earlier studies when dealing with ZnO nanoparticles (Haider et al., 2020, Shukla et al., 2020, Hilal et al., 2021).

Zinc oxide (ZnO) nanoparticles exhibit a notable advantage in terms of environmental concerns, standing out as a promising material with minimal ecological impact (Raha and Ahmaruzzaman, 2022). Unlike certain nanoparticles that raise apprehensions due to their potential toxicity, ZnO nanoparticles are generally regarded as environmentally benign. Their low toxicity is attributed to the fact that zinc, a key component of ZnO, is an essential micronutrient for both plants and animals. Additionally, ZnO nanoparticles possess a high level of biocompatibility, making them less likely to cause adverse effects in living organisms (Mahalakshmi et al., 2020). These characteristics collectively position ZnO nanoparticles as a promising and environmentally friendly option for sustainable pest control in stored wheat.

5. Conclusion

This study explored the impact of green synthesized ZnO-NPs from the extract of scarlet pimpernel (*Anagallis arvensis*) on red flour beetle (*Tribolium castaneum*) in wheat flour. The present study concluded that green synthesized ZnO NPs have good toxicity against *T. castaneum*. Among all concentrations of NPs, 1000 mg/L was found to be more active to control red flour beetle Thus Phyto-fabricated ZnO Nanoparticles can be used as an alternative to synthetic insecticide because it is eco-friendly and effective at the same time to overcome the infestation of *T. castaneum*. While the study provides valuable insights into the insecticidal efficacy of zinc oxide nanoparticles (ZnO NPs) fabricated with *Anagallis arvensis* extracts against *Tribolium castaneum*, there are certain limitations that should be acknowledged. Firstly, the investigation primarily focuses on laboratory conditions, and the extrapolation of these findings to real-world, field conditions may require further exploration. Additionally, the study primarily assesses the acute toxicity of ZnO NPs, and a more comprehensive understanding of their sublethal effects and long-term impacts on non-target organisms is crucial for a holistic evaluation of their environmental safety.

6. Availability of data and material

The data supporting the conclusions of this article are included within the article. Any queries regarding these data may be directed to the corresponding author.

CRediT authorship contribution statement

Adila Maryum: Writing – original draft, Visualization, Investigation, Methodology. **Humaira Yasmin:** Funding acquisition, Writing – review & editing, Methodology, Resources. **Qamar Saeed:** Data curation, Writing – original draft, Visualization, Investigation, Resources. **Ashraf M. Ahmed:** Funding acquisition, Validation, Resources, Project administration, Software. **Simona Mariana Popescu:** Data curation, Writing – review & editing. **Faheem Ahmad:** Conceptualization, Funding acquisition, Writing – review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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