



Efficacy of Zn, Fe and Mg Nano Scale and Bulk Plant Nutrient Foliar Sprays in Biochemical Changes of Blackgram Genotypes under YMV Tolerance

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Authors' contributions

This work was carried out in collaboration among all authors. Author BR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VR and PS managed the analyses of the study. Author PS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Evidence-based synergistic effects of Nanoscale materials, which size typically falls below 100 nm, exhibit novel chemical, physical and biological properties which are different from their bulk counterparts. Here, we report for the first time comparison of nanoscale zinc oxide (n-ZnO), magnesium oxide (n-MgO), and iron oxide (n-FeO) with their respective bulk concentrations on biochemical variability of blackgram genotypes under yellow mosaic virus condition. Oxides of nano scale Zn, Mg and Fe materials were prepared by using a modified sol-gel method. Characterization was performed by using X- ray diffraction (XRD), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM) Analysis and High- Resolution Transmission Electron Microscopy (HRTEM). Average sizes of ZnO, MgO and FeO (78.8, 60.8 and 12.4 nm respectively) and potentials (-36.7,-33.0 and -18.0 mV respectively). A field study was conducted during summer 2018 in split-plot design with two blackgram genotypes as main treatments (TBG-104 and LBG-623) and nine sub treatments (Control, ZnSO₄ @ 0.2 %, n-ZnO @ 200ppm, MgSO₄ @ 0.2 %, n-MgO @ 100ppm, FeSO₄ @ 0.2%, n- FeO @ 200ppm, ZnSO₄ @ 0.2 % + MgSO₄ @ 0.2 % + FeSO₄ @ 0.2 %, n-ZnO @ 200ppm + n- MgO @ 100ppm + n- FeO @ 200ppm) as foliar sprays

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with three replications. YMV infection was observed at 25 DAS. The foliar treatmental sprays were imposed at 30 and 50 DAS.

Results of the field study revealed that combined application of nanoscale treatmental spray ZnO @ 200 ppm + MgO @ 100 ppm + FeO @ 200 ppm resulted higher YMV tolerance interms of high chlorophyll content, total phenols, total proteins and lower total soluble sugars, followed by combined bulk treatmental spray ZnSO₄ @ 0.2% + MgSO₄ @ 0.2% + FeSO₄ @ 0.2% and nano scale Magnesium Oxide @ 100 ppm. Here we concluded that either of these three foliar sprays can be recommended to blackgram crop prone to YMV condition to sustain plant growth and final yields. But, there should be study on the impact of different shape, size of nano particles on plant system and the promotery effects of nano scale Oxides of Zn, Mg and Fe particles at cellular level and transport mechanism in plant system needs to be understood.

Keywords: Nanotechnology -nanomaterials –Zn; Mg; Fe oxides-YMV-blackgram.

1. INTRODUCTION

Nanoparticles are atomic or molecular aggregates posing modified physical–chemical properties compared to the bulk materials and having less than 100 nm measured size in atleast one dimension [1]. The demand for nanoscience and nanotechnology based products has been increasing rapidly in several fields as a solution to an array of critical problems which were not addressed effectively by any other technology or science so far.

Whitefly-transmitted bipartite begomoviruses named as Mungbean yellow mosaic India virus (MYMIV), is the most distinct etiological agent of YMV disease in blackgram in India and other South Asian countries [2].

Research on epidemiological aspects indicates that MYMV disease incidence depends upon the host genotypes, growing seasons and prevailing environmental conditions [3] Certain resistant genotypes are now available to the breeders and farmers but no information is available on the mechanism of disease resistance in these genotypes. Being dependent on metabolic system, plant viruses cause disturbances in the physiology and biochemical components of infected plants [4]. Most of the metabolic changes observed are probably indirect effects of viral infection as a result of interference with various physiological and biochemical processes, besides the transport of water, nutrients, and other substances. Yield losses in blackgram due to this disease are reported to vary from 10 to 100 per cent [5]. Minerals, apart from being a vital part of the plant nutrition, may manifest certain maladies in the plants either through disturbing normal metabolism, physiology and biochemical variability of the plants by favouring or by discouraging the plant pathogens, if in

excess or otherwise deficient. Disturbance in growth regulation results in morphological abnormalities, ranging from a mosaic pattern on leaves and flowers to necrotic spots and streaks to leaf enation and tumours [6].

Currently, nanotechnology is extensively used in modern agriculture to make true the concept of precision agriculture. Nanotechnology includes nano particles having one or more dimensions in the order of 100 nm or less [7]. Nano materials find applications in plant protection, nutrition and management of farm practices due to small size, high surface to volume ratio and unique optical properties [8].

Considerable progress has been made in the recent over the past few years in understanding the mechanisms of disease resistance or susceptibility [9] and it has been reported that resistance to any virus depends on plant metabolism.

However a through insight into morphological, physiological and biochemical basis of YMV tolerance in blackgram is not fully understood. Role of bulk and nano scale micronutrients in imparting tolerance to YMV needs further emphasis. In the present investigation various bulk and nano-scale plant nutrients were evaluated on biochemical basis for improving YMV tolerance in blackgram genotypes.

2. MATERIALS AND METHODS

The present field experiment was conducted at S.V. Agricultural college farm, Tirupati campus of Acharya N.G. Ranga Agricultural University, during summer 2018 which is graphically situated at 13.5°N latitude and 79.5°E longitude, with an altitude of 182.9 m above the mean sea level in the southern Agro- Climatic Zone of Andhra

Pradesh. YMV infection was high and uniform during summer compared to *Rabi*. In summer 2017 at 45 DAS nearly 37.5 per cent of higher ymv infection was noticed compared to *rabi* 2016-17 [3]. Hence the present evaluation was taken up during summer 2018 only where disease pressure is high and reliable data can be generated regarding nutrient interventions in YMV tolerance. Based on the field evaluation for screening blackgram genotypes during *Rabi* 2016-17 and Summer 2017, one highly resistant (TBG-104) and one highly susceptible (LBG-623) genotypes were selected and used in the present field study as well as the concentration of foliar sprays of bulk ZnSO₄, MgSO₄ and FeSO₄ was already standardized [10]. However safer foliar concentrations of nano scale Oxide particles of Zn, Mg and Fe were standardized by the method of treating blackgram seeds with various concentrations of nano scale nutrients in comparison with bulk nutrients and identified appropriate concentrations based on promotory effect on seedling growth under lab conditions of S.V. Agricultural college, Tirupati in 2017.

The present experiment was laid out in a split-plot design with two main treatments (TBG-104 and LBG-623), nine sub treatments (ZnSO₄ @ 0.2%, MgSO₄ @ 0.2%, FeSO₄ @ 0.2% as individual bulk treatment sprays, ZnO @ 200ppm, MgO @ 100 ppm, FeO @ 200 ppm as individual nanoscale treatment sprays and along with combined bulk and nanoscale treatment sprays ZnSO₄ @ 0.2 % + MgSO₄ @ 0.2 % + FeSO₄ @ 0.2 % and Nano scale ZnO @ 200 ppm + MgO @ 100 ppm + FeO @ 200 ppm respectively including control water spray) and replicated thrice.

2.1 Synthesis of Zinc Oxide, Magnesium Oxide and Iron Oxide Nanoparticles

Nanoscale Zinc Oxide particles were prepared using the modified oxalate decomposition technique. Zinc oxalate was prepared by mixing equimolar (0.2 M) solutions each of zinc acetate and oxalic acid. The resultant precipitate was collected and rinsed extensively with double deionized water and dried in air. Oxalate was then ground and decomposed in air by placing it in a preheated furnace for 45 min at 500°C. Magnesium Oxide nanoparticles were prepared by the sol-gel method using magnesium nitrate and sodium hydroxide at room temperature. Then the solution was filtered using filter paper (Whatman No. 1), and hydrated magnesium oxide nanoparticles were dried at

500°C for 3 h. Nano scale Iron Oxide particles were prepared using the sol-gel method. In this method, 1% ferric nitrate (tetrahydrate purified) was mixed with 0.05% sodium hydroxide drop by drop and stirred at 60°C for 3 h. Then, the solution was filtered using filter paper (Whatman No. 1) and dried at 500°C for 2 h. The collected powder was used for characterization.

LBG -623 genotype was severely affected by yellow mosaic virus (YMV) at 25 DAS. No control measures were taken for YMV. Treatments were imposed at 30 DAS and 45 DAS.

2.2 Biochemical Parameters (At 30, 50 DAS)

2.2.1 Total chlorophyll content (mg g⁻¹)

The concentration of total chlorophyll was quantified by reading the optical density at 663 and 645 nm and it was calculated by using the formula given by Arnon [11].

$$\text{Total Chlorophyll (mg g}^{-1} \text{ tissue)} = [20.2 (D_{645}) + 8.02 (D_{663})] \times \frac{V}{1000} \times W$$

where,

V = Volume made up

W = Weight of leaf sample

2.2.2 Total phenol content (g g⁻¹)

The total phenols were determined by AOAC colorimetric method at 650 nm by spectrophotometer [12].

2.2.3 Total soluble sugars (TSS) (g g⁻¹)

Total Soluble Sugars were estimated by Anthrone method given by Sadasivam and Manickam [13]. Total soluble sugars are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green colored product with an absorption maximum at 630 nm.

2.2.4 Total leaf protein content (mg g⁻¹)

Total leaf protein was estimated calorimetrically as per the method developed by Lowry et al. [14] and recording absorbance at 595 nm. Bovine serum albumin was used as standard. Protein content in leaf samples was recorded as µg of protein per g of leaf.

Table 1. Effect of bulk and nano scale zinc, magnesium and Iron nutrients on biochemical parameters of blackgram genotypes

Treatments	Biochemical parameters (at 50 DAS)											
	Total Chlorophyll Content (mg g ⁻¹)			Total phenols (g g ⁻¹)			Total soluble sugars (TSS) (g g ⁻¹)			Total protein (mg g ⁻¹)		
	G ₁	G ₂	Mean	G ₁	G ₂	Mean	G ₁	G ₂	Mean	G ₁	G ₂	Mean
T ₀ :Control (Water spray)	0.630	0.481	0.555	11.250	12.230	11.792	0.270	0.396	0.333	0.715	0.891	0.803
T ₁ :ZnSO ₄ @ 0.2%	0.791	0.535	0.663	11.000	12.097	11.548	0.260	0.384	0.322	0.746	0.761	0.754
T ₂ :Nano scale Zinc Oxide @ 200 ppm	0.768	0.545	0.657	10.483	11.540	11.012	0.224	0.356	0.290	0.758	0.872	0.815
T ₃ :MgSO ₄ @ 0.2%	0.830	0.635	0.733	9.573	10.583	10.078	0.200	0.260	0.230	0.590	0.612	0.601
T ₄ :Nano scale Magnesium Oxide @ 100 ppm	0.800	0.665	0.733	9.483	10.420	9.952	0.201	0.255	0.228	0.599	0.620	0.610
T ₅ :FeSO ₄ @ 0.2%	0.771	0.560	0.665	10.553	11.680	11.117	0.325	0.214	0.270	0.700	0.752	0.726
T ₆ :Nano scale Iron Oxide @ 200 ppm	0.731	0.541	0.636	10.560	11.467	11.013	0.258	0.199	0.228	0.703	0.759	0.731
T ₇ :ZnSO ₄ @ 0.2 % + MgSO ₄ @ 0.2% + FeSO ₄ @ 0.2%	0.856	0.658	0.757	8.250	10.163	9.203	0.205	0.129	0.167	0.555	0.601	0.578
T ₈ :Nano scale Zinc Oxide @ 200 ppm + Magnesium Oxide @100 ppm + Iron Oxide @ 200 ppm	0.891	0.684	0.788	7.983	9.860	8.922	0.184	0.110	0.147	0.492	0.559	0.526
Mean	0.785	0.589		9.904	11.116		0.201	0.292		0.651	0.714	
SEm ±	0.008	0.003	0.008	0.030	0.014	0.090	0.003	0.001	0.009	0.012	0.006	0.019
CD (P = 0.05)	0.022	0.018	0.033	0.197	0.040	0.109	0.020	0.001	0.004	0.034	0.043	0.058

G₁: TBG-104, G₂: LBG-623

2.3 Statistical Analysis

The experimental data were analyzed statistically by following standard procedure outlined by Panse and Sukhatme [15]. Significance was tested by comparing 'F' value at 5 per cent level of probability. Correlation studies were undertaken for biochemical parameters according to the method proposed by Fisher and Yates [16].

3. RESULTS

The results of present investigation revealed Efficacy of Zn, Fe and Mg Nano scale and bulk plant nutrient foliar sprays in biochemical changes of blackgram genotypes under YMV conditions.

3.1 Biochemical Parameters

3.1.1 Total chlorophyll content (mg g^{-1})

Total chlorophyll showed significance variation among genotypes as well as treatments. Interaction effects also varied significantly at 50 DAS. Among the two tested blackgram genotypes, tolerant genotype TBG- 104 showed 24.07 per cent (0.785 mg g^{-1}) higher mean total chlorophyll content compared to susceptible genotype LBG- 623 (0.589 mg g^{-1}) at 50 DAS. All the treatmental sprays showed significantly higher total chlorophyll content compared to water spray control.

The present investigation revealed that among all the foliar treatmental sprays combined application of nano scale Zinc Oxide @ 200 ppm + Magnesium Oxide @ 100 ppm + Iron Oxide @ 200 ppm recorded 41.90 per cent (0.788 mg g^{-1}) higher mean total chlorophyll content followed by combined application of bulk ZnSO_4 @ 0.2% + MgSO_4 @ 0.2% + FeSO_4 @ 0.2% (36.39%) (0.757 mg g^{-1}) compared to water spray (0.555 mg g^{-1}).

Among all the individual foliar sprays nano and bulk Magnesium sprays recorded higher total chlorophyll content compared to other individual sprays at 50 DAS as all the three nutrients play direct role in chlorophyll synthesis. In the present study nano and bulk Iron and Zinc sprays recorded moderate increase in total chlorophyll content compared to water spray

control *i.e.* ranged between 0.636 to 0.665 mg g^{-1} .

3.1.2 Total Phenols (g g^{-1})

Significant variations were observed among genotypes as well as treatments at both stages of crop growth period. Interaction effects also varied significantly at 30 and 50 DAS. Significant increase was noticed in total phenols from 30 to 50 DAS irrespective of treatments among genotypes due to severity of YMV infection at 50 DAS.

Among two blackgram genotypes tested, susceptible one LBG-623 recorded 12.22 per cent (11.116 g g^{-1}) higher mean total phenol content than tolerant genotype TBG-104 (9.904 g g^{-1}) at 50 DAS.

All the treatmental sprays recorded lower total phenol content compared to control water spray. Among all the individual sprays nano scale Zinc Oxide @ 200 ppm, Magnesium Oxide @ 100 ppm, Iron Oxide @ 200 ppm were recorded lower value of mean total phenol content compared to the irrespective bulk treatmental sprays at 50DAS. Similar results were reported by Basavaraj [17] in MYMV infected blackgram plants than in healthy plants by 34% to 94% among treatments.

Among all the treatmental sprays, combined application of nano scale Zinc Oxide @ 200 ppm + Magnesium Oxide @ 100ppm+ Iron Oxide @ 200ppm recorded 24.44 per cent (8.922 g g^{-1}) lower mean total phenol content followed by combined application of bulk ZnSO_4 @ 0.2% + MgSO_4 @ 0.2% + FeSO_4 @ 0.2% (21.96%) (9.203 g g^{-1}) compared to control (11.792 g g^{-1}). However individual sprays of nano and bulk Iron and Zinc sprays did not showed significant effect on total phenol content.

3.1.3 Total Soluble Sugars (TSS) (gg^{-1})

Significance increase in TSS was observed from 30 to 50 DAS irrespective of treatments. TSS showed significant variations between genotypes as well as treatments. Interaction effects also varied significantly in both stages.

Among the two tested blackgram genotypes, susceptible genotype LBG-623 recorded 45.27 per cent (0.292 g g^{-1}) higher mean TSS than tolerant genotype TBG-104 (0.201 g g^{-1}) at 50

DAS. Susceptible genotypes recorded higher TSS compared to tolerant genotypes [18]. Treatments which showed lower TSS consider as best to enhance YMV tolerance. Combination treatmental sprays showed better results compared to other treatments.

Among all the treatmental sprays combined application of nano scale Zinc Oxide @ 200ppm + Magnesium Oxide @ 100 ppm + Iron Oxide @ 200 ppm recorded lower mean TSS of 0.147g g^{-1} followed by combined application of bulk ZnSO_4 @ 0.2% + MgSO_4 @ 0.2% + FeSO_4 @ 0.2% (0.167g g^{-1}).

3.1.4 Total leaf protein(mg g^{-1})

Variability in total leaf protein content of two blackgram genotypes in response to bulk and nano micronutrient (Zn,Mg,Fe) sprays was recorded at 30 and 50 DAS. Significant increase was recorded in total proteins from 30 to 50DAS irrespective of treatments among genotypes.

Total proteins showed significant variation between treatments as well as interaction effects. Among the two tested blackgram genotypes, tolerant genotype TBG-104 recorded 9.67 per cent (0.651 mg g^{-1}) lower mean total protein content than susceptible genotype LBG-623 (0.714 mg g^{-1}) at 50 DAS. Rajitha et al. [18], 2018 reported that genotypes which showed higher protein content were most susceptible to YMC. Susceptible genotypes recorded higher increase in mean total protein content compared to tolerant genotypes from 30 to 50 DAS.

In the present investigation, treatmental sprays maintained less increase in total protein content by enhancing YMV tolerance compared to water spray control. Among the individual treatmental sprays nano and bulk Magnesium treatmental sprays showed better effect on total protein content compared to other bulk and nano micronutrients.

Among all the treatmental sprays combined application of nanoscale Zinc Oxide @ 200ppm + Magnesium Oxide @ 100ppm + Iron Oxide @ 200ppm recorded 34.50 per cent (0.526 mg g^{-1}) lower mean total protein content followed by combined application of bulk ZnSO_4 @0.2% + MgSO_4 @ 0.2% + FeSO_4 @ 0.2% (28.02%) (0.578 mg g^{-1}).

4. DISCUSSION

Total chlorophyll content indicate a loss of total chlorophyll content due to virus infection in blackgram plants. Both nano and bulk Iron and Zinc recorded better results due to Iron deficiency in plants causes a reduction in chlorophyll and the other anthocyanin contents [19] and Zinc deficiency disrupted the chlorophyll synthesis. Increased chlorophyll contents are due to Zinc which acts as a structural and catalytic component of proteins, enzymes and as co-factor for normal development of pigment biosynthesis [20].

In the present study Higher YMV tolerance observed with lower increase in phenol content which considered better treatmental sprays effects to sustain higher growth due to High amount of total phenols in the resistant types were accompanied by increased activities of polyphenol oxidase and peroxidase, resulting in increased oxidation of phenolic substances to form more toxic quinines and others oxidative products which might aid to combat the pathogen in the resistant host.

Combined application of Zinc, Magnesium and Iron in nanoscale or bulk micronutrients were effective in restricting whitefly transmitted YMV infection by maintaining low TSS content and higher phenols in blackgram leaves. Similar results were concluded by Sahu, [21] in soybean.

5. CONCLUSION

From the study combined spray of nano scale nutrients followed by bulk nutrimental sprays showed better results compared to other individual foliar sprays. The individual foliar sprays viz., nano scale Magnesium Oxide @ 100 ppm, nano scale Zinc Oxide @ 200 ppm and bulk MgSO_4 @ 0.2% were found better for yield enhancing higher biochemical parameters viz., chlorophyll content, total phenols, total proteins and reducing TSS. Here we concluded that either of these three foliar sprays viz., nano scale treatmental spray ZnO @ 200 ppm + MgO @ 100 ppm + FeO @ 200 ppm, bulk treatmental spray ZnSO_4 @ 0.2% + MgSO_4 @ 0.2% + FeSO_4 @ 0.2% and nano scale Magnesium Oxide @ 100 ppm can be recommended to blackgram crop prone to YMV condition to sustain plant growth and final yields. But, there should be study on the impact of different shape, size of nano particles on plant system and the

promotory effects of nano scale Oxides of Zn, Mg and Fe particles at cellular level and transport mechanism in plant system needs to be understood. The results thus obtained needs to be tried/verified on paddy fallows in relay cropping system under zero tillage where large area of blackgram is planted in Andhra Pradesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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