ACTIVITY OF SOME NANO PARTICLES IN CONTROLLING BEAN YELLOW MOSAIC VIRUS ON FABA BEAN

BY

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ABSTRACT

Nanotechnology has emerged as a new potential powerful tool to control viral plant diseases. This study was carried out to evaluate the effectiveness of four nanoparticles namely silver nanoparticles (AgNPs), chitosan nanoparticles (ChiNPs), chitosan-silver nanocomposites (Chi-AgNPs) and chitosan-salicylic acid nanocomposites (Chi-SalNPs) in managing *Bean yellow mosaic virus* (BYMV) on faba bean plants from the plant-virus-vector interaction side. The antiviral capability was evaluated as a foliar application, seed soaking and seed /foliar combination methods. The efficiency of tested nanoparticles on virus acquisition and transmission by its aphid vector was investigated as well as potential treatments to affect the vector aphid population dynamics. The results indicated that all tested nanoparticles significantly reduced the virus infectivity and accumulation content in treated plants notably when applied as foliar application and seed/foliar combination. Nano-silver was exhibited high curative viricidal activities to inactivate BYMV when applied 48 h post-virus inoculation. The disease occurrence was entirely inhibited with AgNPs rate as low as 100 mg. l^{-1}, whereas the infectivity was completely inhibited when plants were exposed to 200 mg.l^{-1} 24 h before-virus inoculation. However, ChiNPs, Chi-AgNPs and Chi-SalNPs completely inhibited the virus infectivity at 400 mg.l^{-1} when applied as a foliar protective method. Seed soaking applications of Chi-AgNPs and Chi-Sal NPs were the most effective treatments in reducing the virus infectivity followed by ChiNPs and AgNPs. Interestingly, transmission electron microscope illustrated that AgNPs proved to be highly bio-reactive by binding to the virus particles, while the ChiNPs were found to affect the virus particle's integrity by producing defective and incomplete BYMV particles, suppressing their replication and accumulation within the plant tissues. Moreover, both AgNPs and ChiNPs were significantly found to upregulate the pathogenesis-related gene (PR-1) and promote the protein profile in treated plants irrespective of concentration. The mRNA of PR-1 gene was remarkedly accumulated in treated plants reaching its maximum with 12.06 and 16.22 fold change at 200 mg.l^{-1} AgNPs and 400 mg.l^{-1} ChiNPs dosage rates respectively. The ability of tested nanoparticles to trigger defense-related oxidizing enzymes was also examined. The higher activity of phenylalanine ammonolyses (PAL) and polyphenol oxidase (PPO) was recorded in faba bean plants treated with Chi-SalNPs and ChiNPs, while the lowest response was noted with

all tested AgNPs rates. Peroxidase (PO) activity was significantly prompted with all tested nanoparticles reaching its maximum with AgNPs (at 250 $mg.l^{-1}$) followed by Chi-AgNPs (at 300 $mg.l^{-1}$) Chi-SalNPs (at 400 $mg.l^{-1}$) and ChiNPs (at 250 $mg.l^{-1}$). Furthermore, the total phenols were remarkably promoted for 30 days in response to ChiNPs, Chi-AgNPs and Chi-SalNps applied as seed soaking at 400 $mg.l^{-1}$, compared to untreated control. Importantly, exposure of aphids to AgNPs-treated plants before virus acquisition reduced BYMV acquisition and transmission efficiency by 40.65% to 100 % at 24 h post-application depending on the AgNPs dosage. Further, the virus acquisition was reduced for 10 day-post treatments by 6.87% up to 79.64% depending on the dosage rate. On the other hand, the virus transmission by aphids in faba bean plants treated with tested nanoparticles 24 h before the biological inoculation of BYMV by viruliferous aphids was observed. The complete reduction in virus transmission was obtained with AgNPs at a low rate of 150 $mg.l^{-1}$ dosage, followed by Chi-AgNPs and ChiNPs at 250 and 300 $mg.l^{-1}$ dosage rates respectively. Moreover, all tested nanoparticles reduced the aphid population density after 30 days of application on treated faba bean plants. ChiNPs (400 $mg.l^{-1}$), Chi-AgNPs (400 $mg.l^{-1}$) and AgNPs (300 $mg.l^{-1}$) were the most effective treatments in reducing the aphid population by 96.64%, 95.89% and 92.15, respectively. Meanwhile, Chi-SalNPs reduce the aphid population by 80.56 % compared to untreated control.

Finally, these results confirm that the constructed nanoparticles are powerful and promising antiviral agents to manage BYMV disease. This study also provides the first report on the deterring activity of nanomaterials on plant virus acquisition and transmission by its insect vector. Simultaneously, the tested nanoparticles can affect the vector feeding behavior and alter virus-aphid transmissibility, suggesting that it may contribute to alleviating the natural disease occurrence and virus transmission under field conditions.

ABBREVIATIONS

θ	Theta angel
Δ	Delta
A°	Angstrom
A_{405}	Absorbtion at 405 nm
AAP	Acquisition access period
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
AMV	*Alfalfa mosaic virus*
ANOVA	Analysis of variance
bp	Base pair
BYMV	*Bean yellow mosaic virus*
°C	Degree celsius
CABI	Centre for Agriculture and Bioscience International
C+ve	Positive control
C-ve	Negative control
ChiNPs	Chitosan nanoparticles
Chi-AgNPs	Chitosan-silver nanocomposites
Chi-SalNps	Chitosan-salycalic acid nanocomposites
cm	Centimeter
cm^2	Centimeter square
Conc.	Concentrations
CP gene	Coat protein gene
cv.	Cultivar
Ct. value	Cycle threshold
DI	Disease incidence
DS	Disease severity
dw	Distilled water
DAS-ELISA	Double antibody sandwich – Enzyme-linked Immunosorbent Assay
F.A	Foliar application
FAO	Food and agriculture organization of the united nations
g	gram
Gov.	governorate
h	hour (s)
HR-TEM	High resolution- transmission electron Microscope
gfw	gram fresh weight
kb	Kilobase
kDa	Kilodalton
LA	Leaf area
LL	Leaflet length

LW	Leaf width
LSD	Least significant difference
M	Molar (concentration)
mA	Milliampere
mg	Milligram
$mg.l^{-1}$	Milligram per liter
min.	Minute
µl	Microliter
ml.	Milliliter
mmol	Milimole
N	Normality (concentration)
ng	Nanogram
No.	Number
nmol	Nanomole
OD	Optical density
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
nm	nanometer
PAL	Phenylalanine ammonia-lyase
PO	Peroxidase
PPO	Polyphenol oxidase
pH	Potential of Hydrogen
PR-proteins	Pathogenesis-related proteins
PSbMV	*Pea seed-born mosaic virus*
Red.	Reduction
RH	Relative humidity
rpm	Revolutions per minute
RT-PCR	Reveres transcription-polymerase chain reaction
qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
SA	Salicylic acid
Sec.	Second
SDS	Sodium dodecyl sulfate
SSA	Seed soaking application
SFA	Seed/ foliar combinations
TEM	Transmission Electron Microscope
Treat.	Treatments
TPC	Total phenolic content
v/v	Volume per volume
w/v	Weight per volume
xg	(X) Earth's gravitational force
XRD	X-ray diffraction

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