ISSN: 0067-2904

Control of Gray Mold on Tomato plants by Spraying *Piper nigrum* and *Urtica dioica* Extracts under Greenhouse Condition

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Abstracts

Field experimented were examined the effects of *Piper nigrum* and *Urtica dioica* extracts on the gray mold disease in tomato that caused by *Botrytis cinerea*. To evaluate the inducing resistance of these extracts, many treatments were sprayed on tomato leaves, including methanolic and aquatic extracts, Silver nano particles biosynthesis (AgNPs) and water as (control).

The results indicated that the resistance of tomato plants was increased when tomato plant sprayed first with Methanolic *P. nigrum* extracts and after 4 hours sprayed with *B. cinerea*. Also, spraying with methanolic and aquatic AgNPs *P. nigrum* extract were reduced gray mold disease. These results were showed that *P. nigrum* AgNPs treatment reduced the gray mold of tomato leaves because of the activities of total phenolic compounds which was infected with *Botrytis cinerea.*

Keywords: Tomato, gray mold, induced resistance, Disease severity.

السيطرة على مرض العفن الرمادي على نبات الطماطة برشها بمستخلص الفلفل الاسود والقراص تحت ظروف البيت الزجاجي

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الخلاصة

اجريت تجارب حقلية لدراسة تأثير مستخلص الفلفل الاسود والقراص في السيطرة على مرض العفن الرمادي على نبات الطماطة والذي يسببه فطر *Botrytis cinerea*. تضمنت الدراسة قياس تأثير زيادة المقاومة من خلال رش اوراق النباتات بعدة محاليل والتي شملت المستخلص المائي والميثانولي ومستخلصات نانوية للنباتين، فضلا عن استخدام الرش بالماء فقط لمعاملة السيطرة. اظهرت النتائج ان رش اوراق الطماطة بالمستخلص الميثانولي النانوي للفلفل الاسود ومن ثم رشت نفس الاوراق وبعد اربعة ساعات بفطر *B. cinerea* قد زاد من مقاومة النبات للمرض. كذلك كان لرش الاوراق بالمستخلص المائي والميثانولي النانوي للفلفل الاسود الاثر الكبير في تقليل من شدة الاصابة بمرض العفن الرمادي. اوضحت هذه النتيجة ان معاملة الرش بالمستخلص الميثانولي النانوي للفلفل الاسود له تأثير في السيطرة على مرض العفن الرمادي في الطماطة، اذ قد يعود تأثير ذلك الى مجموع المواد الفينولية الموجودة في المستخلص والتي زادت من استحثاث مقاومة الاوراق المصابة بالفطر *B. cinerea*.

Introduction:

Tomato plant (*Solanum lycopersicum* L.) is infected with many fungi and bacterial diseases, which caused a heavy loss to the crop. Tomato is susceptible to gray mold disease although some of the

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tomato cultivars showed numerical resistance. Diseases control caused by *B. cinerea* was released by spraying systemic fungicides as biocontrol agents, although the using of chemical fungicides has faced many obstacles like increasing in community worry regarding infection with fungicidal residues, with increasing the resistance in the pathogen residents [1]. The most effective agents must be safe and natural, with an effective alternative to the disease without using any fungicides.

Botrytis cinerea can cause a characteristic symptomatology named Botrytis gray mold. This fungus has a very wide host range that includes many vegetables crops. Once established it is difficult to control and it may be present in greenhouse crops for all the year, causing serious reduction in yield. Severe infection of stems can often kill the plants. Among alternative methods of gray mold control, the use of natural compounds as plant extracts, which can be characterized by lack of toxicity for humans and environment, selectivity, biodegradable activity and a great variety of chemical composition, with a large variety of secondary metabolites, most of them not yet studied in correlation with their fungicidal action [2].

The nanotechnology becomes more interesting to many scientists, that was because of different properties for the materials at nano-level. The use of silver nanoparticles as ant bioagents are making their production more economical. Meanwhile silver displays different styles of inhibitory act to microbial [3]. Silver nano-particles (AgNPs) may be useful for controlling many pathogens because of the safer way compared with fungicides. Silver is known to affect many biochemical processes in the microbial including the changes in routine functions and plasma membrane permeability [4]. The AgNPs also prevent the expression of ATP production associated proteins [5]. Briefly, the precise mechanism of bio molecules inhibition is to come understood [6].

Thus, use of AgNPs has been measured an alternative and effective approach which is eco-friendly to controlling the pathogenic microbes [7, 8, 9]. This work was conducted to evaluate the effect of spraying black pepper (*Piper nigrum*) and Nettle (*Urtica dioica*) (Methanolic and Aqueous) extracts with and without silver nanoparticles (AgNPs) on suppressing the development of the tomato gray mold disease.

Materials and Methods

Collection of *Botrytis cinerea* L.:

Botrytis cinerea was obtained from state culture for Agric., Res, Ministry of Agriculture, Baghdad-Iraq.

Collection of plant samples:

Plant dried fruits black pepper (*Piper nigrum*) was obtained from the local market and dry leaves of *Urtica dioica* were obtained from the Botanical Garden of the College of Science, University of Baghdad. The plants were powdered after air derided and kept in sterile universal bottles at 4°C.

Plant extracts preparation:

Aqueous extracts:

The dried *P. nigrum* and *U. dioica* with distilled water were mixed in a ration of 1:10 (100 g in 1 L water), with heat for 4 hours. After cooling, the extracts filtered by Whatman No.1 filter paper. The filtrate was the crude extract which kept at 4ºC.

Methanol extracts preparation

In this method, 10 g of ground plant sample transferred into the Soxhlet extractor using 200 ml of methanol for 24h. The extracts were filtered by Whitman filter paper No. 42 (125 mm) and concentrated by rotary evaporator (Laborota 4000, SN 090816862, Germany) with a water bath at 40°C [10]. Then kept in sterile universal bottles at 4ºC.

Biosynthesis of silver nanoparticles:

Aqueous solution and methanol extracts and (1 mM) of $AgNO_3$ were used for the synthesis of AgNPs. Plant extract (5 ml) added into 95 ml of solution of 1 mM $AgNO_3$ for reduction into Ag^+ ions. In a typical biosynthesis of AgNPs the plant extract (1.5 ml) was added to 30 ml of 10^{-3}M $AgNO_3$ aqueous solution in a 250 ml Erlenmeyer flask on a water bath at 75°C for one hour. The formation of AgNPs confirmed by spectrophotometric determination. Also, the reduction of $AgNO_3$ to Ag^+ was confirmed by the color change from colorless to brown. The fully reduced solution centrifuged at 5000 rpm for half an hour. The supernatant liquid discarded and the pellet obtained re-dispersed in deionized water. two to three times were the centrifugation process repeated to wash the surface of the AgNPs [11].

Optical property:

The optical property of silver nanoparticles was detected by Ultraviolet-visible spectrophotometer (UV/Vis) refers to absorption spectroscopy in the ultraviolet-visible spectral region. The samples were adjusted by UV-VIS double beam spectrophotometers.

Different concentrations of plant extracts:

Plant extracts solutions were prepared by mixing 1 g from each dried extract with 10 mL H_2O, and then it was sterilized with a membrane filter (0.22 µm). Then many concentrations of 10 mg/ ml were set by adding known volume from the stock solution with distal water.

The abbreviations of the treatments:

Abbreviations	***Details***
MPB	Tomato Plant sprayed first with Methanolic *P. nigrum* extracts and after 4 hours the same plants were sprayed with *B. cinerea*.
AMPB	Tomato Plant sprayed first with AgNPs Methanolic *P. nigrum* extracts and after 4 hours the same plants were sprayed with *B. cinerea*.
QPB	Tomato Plant sprayed first with Aqueous *P. nigrum* extracts and after 4 hours the same plants were sprayed with *B. cinerea*.
AQPB	Tomato Plant sprayed first with AgNPs Aqueous *P. nigrum* extracts and after 4 hours the same plants were sprayed with *B. cinerea*.
MUB	Tomato Plant sprayed first with Methanolic *U. dioica* extracts and after 4 hours the same plants were sprayed with *B. cinerea*.
AMUB	Tomato Plant sprayed first with AgNPs Methanolic *U. dioica* extracts and after 4 hours the same plants were sprayed with *B. cinerea*.
QUB	Tomato Plant sprayed first with Aqueous *U. dioica* extracts and after 4 hours the same plants were sprayed with *U. dioica*.
AQUB	Tomato Plant sprayed first with AgNPs Aqueous *U. dioica* extracts and after 4 hours the same plants were sprayed with *U. dioica*.
MP	Tomato Plant sprayed with Methanolic *P. nigrum* extracts only.
AMP	Tomato Plant sprayed with AgNPs Methanolic *P. nigrum* extracts only.
QP	Tomato Plant sprayed with Aqueous *P. nigrum* extracts only.
AQP	Tomato Plant sprayed with AgNPs Aqueous *P. nigrum* extracts only.
MU	Tomato Plant sprayed with Methanolic *P. nigrum* extracts only.
AMU	Tomato Plant sprayed with AgNPs Methanolic *U. dioica* extracts only.
QU	Tomato Plant sprayed with Aqueous *P. nigrum* extracts only.
AQU	Tomato Plant sprayed with AgNPs Aqueous *P. nigrum* extracts only.
B	Tomato Plant sprayed with *B. cinerea* only.
Control	Tomato Plant sprayed with distilled water only.

Laboratory experiments.

Plant extracts solutions were used to evaluate their antagonistic activity against *B. cinerea* each extract was added to PDA medium at 10, 20, 30 ml / L. Disc of 5 mm diameter from actively growing culture of *B. cinerea* was placed at the center of petri dishes, each test was replicated three times and inoculation with *B. cinerea* only served as control. Diameter of the pathogen was measured after 7 days of inoculation at 24 C^o. The percent mycelia growth inhibition was calculated using the following equation:

Growth Inhibition = (A – B / A) X 100

A = Diameter of fungal colony (mean) in control.

B = Diameter of fungal colony (mean) in treatment.

Field experiments:

Filed experiment for Induced resistance and biological control:

Induced resistance and biological control in tomato plant against gray mold using black pepper (*P. nigrum*) and nettle (*U. dioica*) methanolic and aqueous extracts with and without silver nanoparticles (AgNPs). This experiment was carried out in green house in plant protection directorate. Tomato seed were planted (20g pore) in storefront, seedling was then transplanting in plastic house (500 m^2) after 35 days, treatments were distributed according to (RCBD) design with three replicates for each treatment and 10 plants for each replicate.

Tomato plants at the flowering stage were sprayed with solutions at 10, 20, 30 ml / L, then inoculated by spore suspension of *B. cinerea* (1 X 10^3 spore/ml).
Disease severity was determinate after 14 days of inoculation according to (Yildiz, 2000), by using the disease scale (1 = 5%, 2 = 25%, 3 = 50%, and 4 = infected leaf area about 75%-100%).

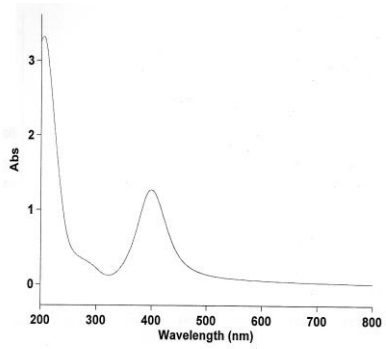
$$\text{Disease severity} = \frac{\Sigma (\text{scale} \times \text{number of infected leaves}) \times 100}{\text{total number of leaves} \times \text{highest scale}}$$

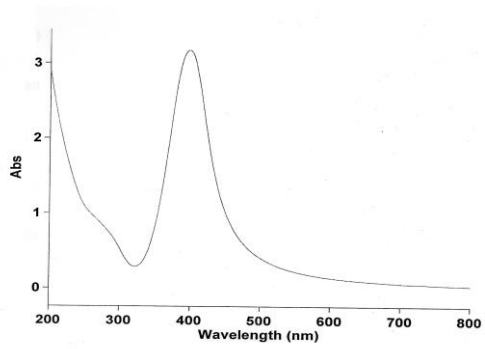
Measurements of Total Phenols accumulation (TPO)

Total phenolic content of leaf extracts was determined using the method of [12].

Results and Dissection

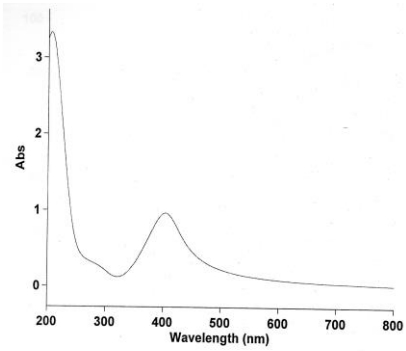
Reduction Ag^+ into AgNPs during contact to plant extracts were observed around 620 nm of plant extracts Figures-(1, 2)**.**

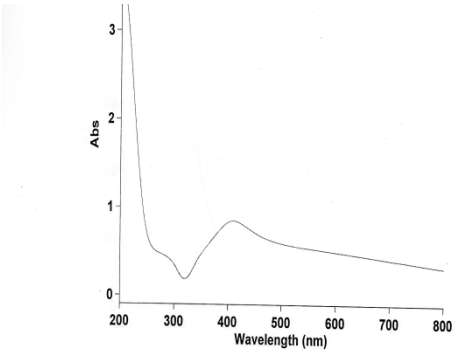




***P. nigrum* and AgNPs (aqueous)** ***P. nigrum* and AgNPs (methanolic)**

Figure 1-UV-vis spectra of P. nigrum extracts by methanolic and aqueous solvents.





***U. dioica* and AgNPs (aqueous)** ***U. dioica* and AgNPs (methanolic)**

Figure 2-V-vis spectra of *U. dioica* extracts by methanolic and aqueous solvents.

In this study Silver nanoparticles have been biosynthesized. By varying the processing conditions, the diameter of the silver nanoparticles was between ~ 70nm to ~ 90nm for aqueous and methanolic extracts for both plants under study.

Optical properties AgNPs are sensitive to several factors such as agglomeration state, concentration, shape, size, and refractive index near the AgNPs surface; which produce UV/vis spectroscopy a valuable tool for characterizing, identifying. But there is a relationship between the optical absorption spectrum of AgNPs caused by surface plasmon absorption and their sizes. The plasmon resonance surface is the coherent excitation of the electrons in the conduction band. For the larger particles (several tens of nanometers) the excitation of the resonance absorption surface can occur in the visible light region (390 - 420 nm for AgNPs). The result presents the wavelength dependence of the ablation efficiency in the extracts system (methanolic or aquatics) in terms of self-

absorption in which colloidal particles generated absorb subsequent. On the behalf of UV-vis data, it was cleared that AgNPs biosynthesized reduces metal ions [13].

Fungicide activity:

The result in Table-1 presented the fungicide activity of two plant extracts solutions against *B. cinerea*. The fungicide activity was fit in all the solutions understudy, although there were significant differences between nano-extracts effectiveness in inhibiting fungi *B. cinerea*. Also, the nano-solutions was found to vary with the specific plant extracts as well as the synthesis of AgNPs preparation. Besides, the result inducted that methanol *P. nigrum* AgNPs was the effectiveness in inhibiting fungi *B. cinerea*, it did not show any visible antifungal activity at 30 mg/L, except for the 10-20 mg/L which the inhibition zone diameter was 72-88 mm as in shown in (Table-1).

Table 1-Bio-fungicide activities of *P. nigrum* and *U. dioica* extracts with and without AgNPs on *B. cinerea* growth.

	Treatment	concentration Extracts			Average
		10ml/L	20ml/L	30ml/L	
		Inhibition rate %			
1	Methanolic *P. nigrum* extract (MP)	50	58	70	59.33
2	Methanolic *P. nigrum* AgNPs (AMP) extract	72	88	100	86.67
3	Aqueous *P. nigrum* extract (QP)	44	51	67	54.00
4	Aqueous *P. nigrum* AgNPs (AQP) extract	70	80	95	81.67
5	Methanolic *U. dioica* extract (MU)	40	51	64	51.67
6	Methanolic *U. dioica* AgNPs (AMU) extract	68	83	94	81.67
7	Aqueous *U. dioica* extract (QU)	45	52	63	53.33
8	Aqueous *U. dioica* AgNPs (AQU) extract	65	76	91	77.33
9	Control	0.00	0.00	0.00	0.00

LSD (P = 0.05) for Treatment = 3.04, for Concentration = 1.46, for Treatment X Concentration = 5.27.

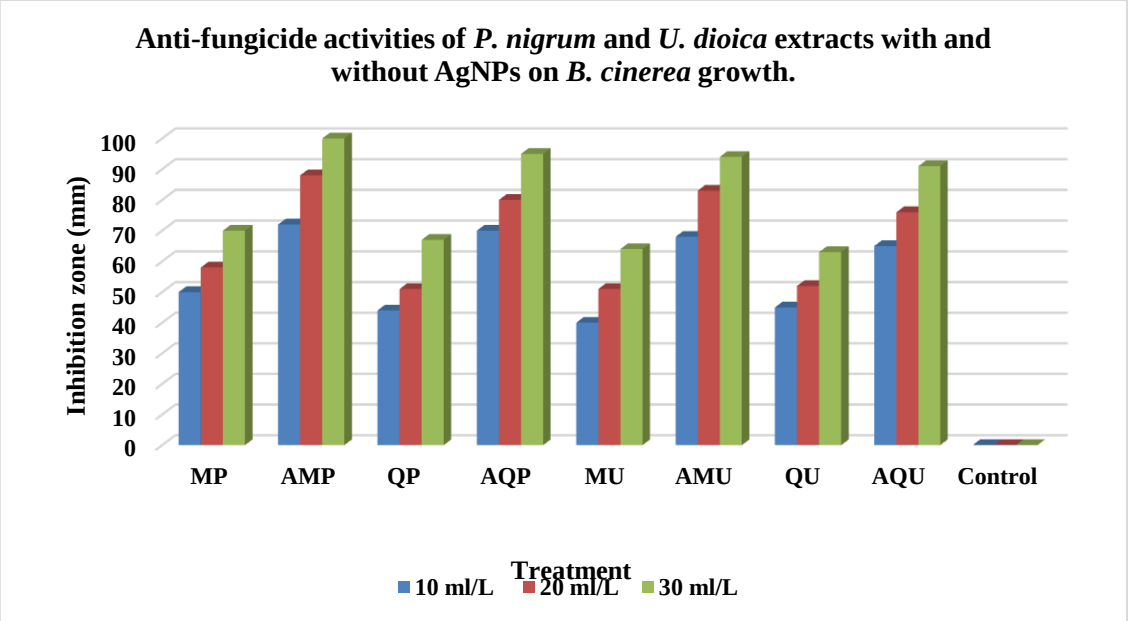


Figure 3-Anti-fungicide activities of *P. nigrum* and *U. dioica* extracts with and without AgNPs on *B. cinerea* growth.

These two plant extracts may contain active compounds which were responsible at least in part for the fungicide activity Figure-3. The findings results are in agreement with Jadou and Al-Shahwany [14], when they exposed that all nano-extracts have a highly effectiveness in inhibiting against some pathogens in relation with concentrations of the methanolic and aqueous extract solutions. Also, they

mention that concentration of the nano-extract was a critical factor for biological activity. Besides, they reported that the AgNPs biosynthesized increased the secondary compound percentage in the solutions, and perhaps, it is the reason for increasing antimicrobial effects compared with other solutions without AgNPs. Also, these results concurred with [15,16], when they found that the inhibitory activities of plant extracts depend on a many factors such as solubility in different organic solvents, geographical conditions, chemical composition, harvest period, extraction methods as well as the test pathogens.

Induced resistance in tomato plant against gray mold

The disease severity of *P. nigrum* and *U. dioica* extracts solutions against *B. cinerea* were summarized in Table-2. The disease severity for the AMP and AQP were the lowest than other solutions, which were 10 and 13%, respectively. While the average for control was 75%. The statistical analysis showed no significant differences at the level of probability ($P \leq 0.05$) between AMP and AQP. Besides, the more active concentration was 30% for all solutions (Figure-4).

Table 2-Induced resistance in tomato plant against gray mold using *P. nigrum* and *U. dioica* extracts with and without AgNPs in greenhouse

Treatment	concentration Extracts			Average
	10ml/L	20ml/L	30ml/L	
	Disease severity (%)			
Methanolic *P. nigrum* extract (MP)	30	25	19	24.67
Methanolic *P. nigrum* extract + AgNPs (AMP)	24	20	10	18.00
Aqueous *P. nigrum* extract (QP)	34	32	22	29.33
Aqueous *P. nigrum* extract + AgNPs (AQP)	25	21	13	19.67
Methanolic *U. dioica* extract (MU)	38	29	23	30.00
Methanolic *U. dioica* extract + AgNPs (AMU)	27	23	15	21.67
Aqueous *U. dioica* extract (QU)	37	30	24	30.33
Aqueous *U. dioica* extract + AgNPs (AQU)	28	24	15	22.33
Control	76	75	74	75.00

LSD (P = 0.05) for Treatment = 1.99, for Concentration = 1.10, for Treatment X Concentration = 3.44

Each number represents the rate of three replicates

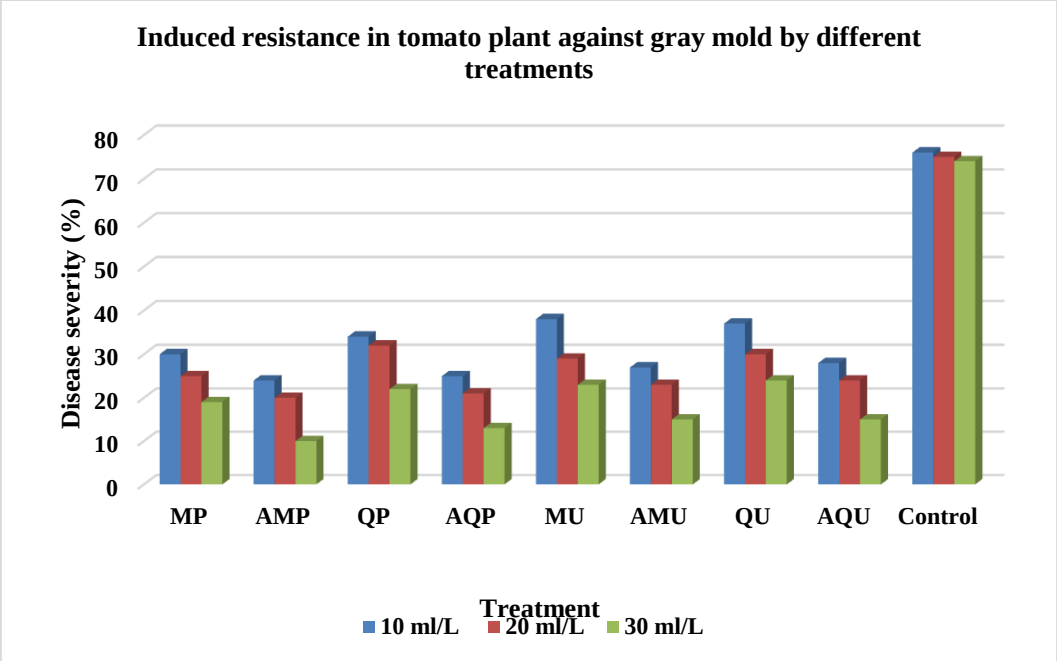


Figure 4-Induced resistance in tomato plant against gray mold using *P. nigrum* and *U. dioica* extracts with and without AgNPs in greenhouse

AgNPs with extracts solutions displayed excellent inhibition on gray mold Figure-4. These extracts might be natural ant-fungi for the treatment diseases could be useful in understanding the traditional cures and current medications [17]. Their mechanism of action appears to be predominantly on the fungal cell membrane, blocking the membrane synthesis; disrupting its structure causing leakage and cell death; fungal proliferation and cellular respiration, inhibition of the spore germination [18].

Biological control of gray mold:

The obtained results in Table-3 show that the interaction between treatments and their concentration significantly affected the disease severity of *P. nigrum* and *U. dioica* extracts solutions against *B. cinerea*. However, AMP solution recorded the highest reduction in disease severity (85%) at 30 ml/L against gray mold.

Table 3-Biological control of gray mold by using *P. nigrum* and *U. dioica* extracts in greenhouse.

Treatment	concentration Extracts			Average
	10ml/L	20ml/L	30ml/L	
	Disease severity (%)			
Methanolic *P. nigrum* extract (MP)	35	29	24	29.33
Methanolic *P. nigrum* extract + AgNPs (AMP)	23	21	15	19.67
Aqueous *P. nigrum* extract (QP)	37	30	27	31.33
Aqueous *P. nigrum* extract + AgNPs (AQP)	30	24	18	24.00
Methanolic *U. dioica* extract (MU)	41	35	27	34.33
Methanolic *U. dioica* extract + AgNPs (AMU)	29	25	20	24.67
Aqueous *U. dioica* extract (QU)	40	34	29	34.33
Aqueous *U. dioica* extract + AgNPs (AQU)	30	26	22	26.00
Fungicide	15	16	14	15.00
Control	81	84	82	82.33

LSD (P = 0.05) for Treatment = 1.71, for Concentration = 0.94, for Treatment X Concentration = 2.97

In addition, the biological control result of gray mold shows the correlation between treatments and their concentrations, especially AgNPs treatments. Practically, since sprayed with the methanolic and aquatic AgNPs solutions of *P. nigrum* which can use as a biological control again (Figure-5).

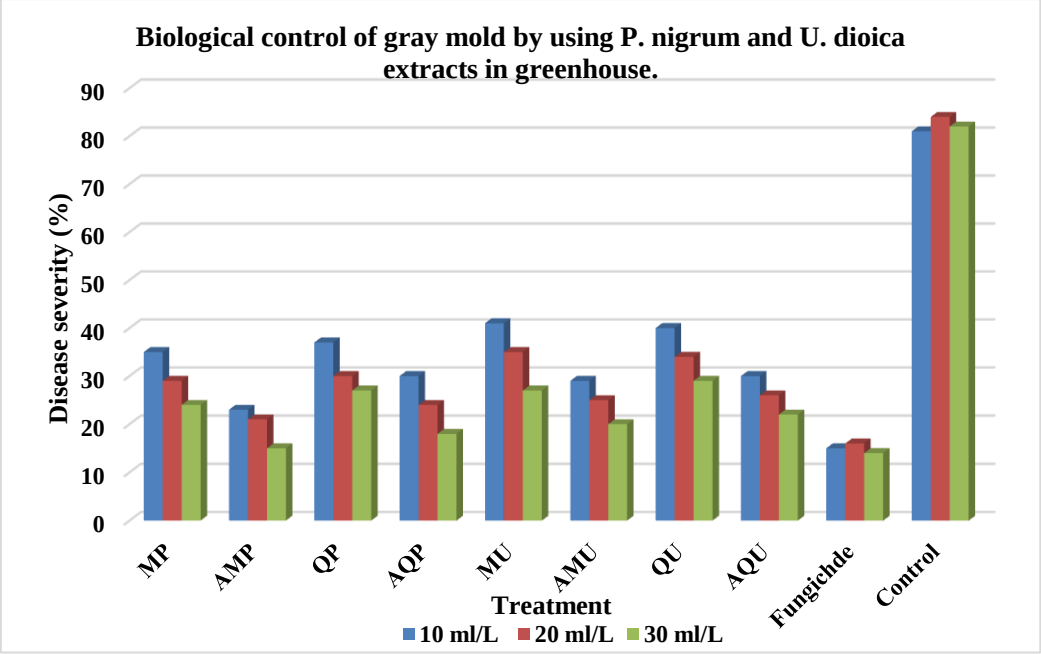


Figure 5-Biological control of gray mold by using *P. nigrum* and *U. dioica* extracts in greenhouse.

Total Phenols (TPO) accumulation

This provided preliminary evidence that the interaction between treatments and their concentration significantly affected of TPO accumulation in leaves, which increased by increased concentration.

TPO accumulation in tomato leaves after sprayed with *p. nigrum* extracts.

Results presented in Table-4, the TPO in tomato leaves after spraying with different concentrations of *P. nigrum* extracts. The TPO accumulation was significantly different among the interaction between the treatments and their concentration. In this study, the highest TPO was (23.67 µg/g fresh weight) recorded by 30 ml/L AMPB treatment, while the lowest TPO (9.40 µg/g fresh weight) was observed in Control treatment. Also, the result of interaction between concentrations and days after treatments were significantly affected of TPO, especially at 30 ml/L concentrated which was (19.09 µg/g fresh weight), while the lowest TPO was (9.92 µg/g fresh weight) recorded at 10 ml/L concentration (Figure-6).

Table 4-Biological activity of *P. nigrum* extracts at different concentrations on TPO (µg/g fresh weight) accumulations after treatments

Treatment	Extract concentration			Average
	10 ml / L	20 ml / L	30 ml / L	
MPB	12.22	15.54	18.42	15.39
AMPB	16.86	22.27	23.67	20.93
QPB	11.82	15.47	18.01	15.10
MP	10.45	10.72	11.00	10.72
AMP	11.36	11.68	11.84	11.63
QP	10.27	10.58	10.91	10.59
B	11.11	11.2	11.23	11.18
CON	9.40	9.49	9.42	9.44
Average	11.69	13.37	14.31	

LSD for treatments = 2.3, for concentration = 1.2, for Treatments X concentration = 3.9

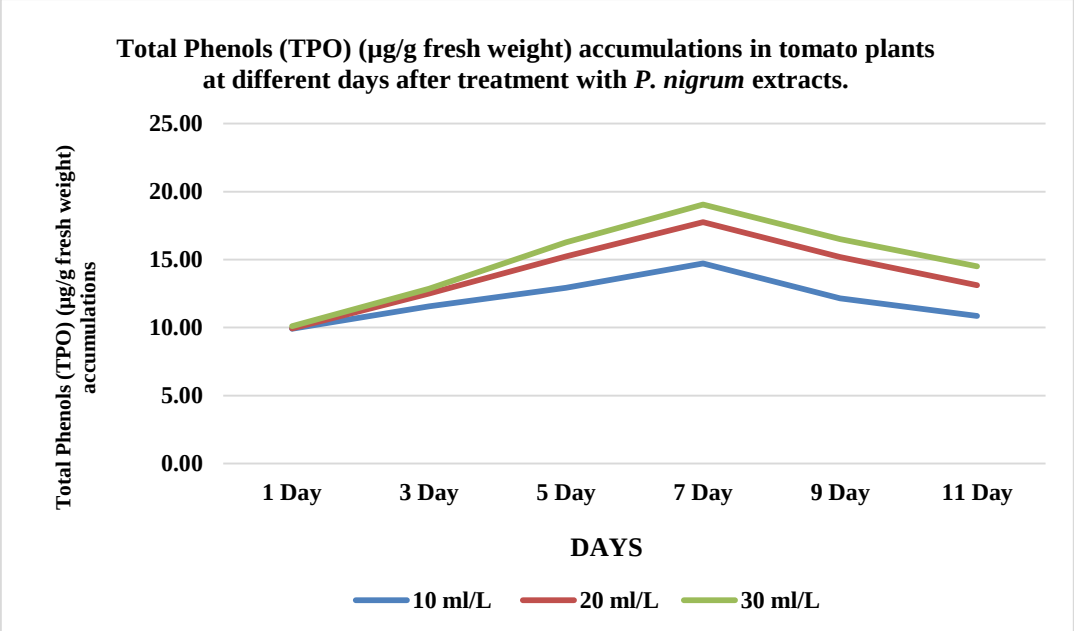


Figure 6-Total Phenols (TPO) (µg/g fresh weight) accumulations in tomato plants at different days after treatment with *P. nigrum* extracts.

Total Phenols (TPO) accumulation in tomato leaves after sprayed with *U. dioica* extracts.

The Results in Table-5 indicated that the TPO was significantly increased. After spray Tomato leaves with different concentrations of *U. dioica* extracts compared to control. However, the highest TPO (20.63 µg/g fresh weight) was recorded by 30 ml/L AMUB treatment, while the lowest TPO (9.26 µg/g fresh weight) was observed in control treatment.

As a result, in Figure-7 showed significant changes occurred in TPO by increasing along the eleven days after treatment. The interaction between concentrations and days after treatment were significantly affected of TPO especially at 30 ml/L con. Which recorded the highest TPO (16.57 µg/g fresh weight), while the lowest TPO (9.93 µg/g fresh weight) was recorded at 10 ml/L concentration.

Table 5-Biological activity of *U. dioica* extracts at different concentrations on Total Phenols (TPO) (µg/g fresh weight) accumulations after treatments.

Treatment	Extract concentration			Average
	10 ml / L	20 ml / L	30 ml / L	
MUB	12.22	13.65	14.08	13.32
AMUB	13.55	19.61	20.63	17.93
QUB	11.56	13.22	14.17	12.98
MU	10.1	10.58	10.7	10.46
AMU	10.97	11.35	11.42	11.25
QU	10.14	10.19	10.46	10.26
B	11.24	11.12	11.29	11.22
CON	9.26	9.43	9.35	9.35
Average	11.13	12.39	12.76	

LSD for treatments = 2.1, for concentration = 1.1, for Treatments X concentration = 3.7

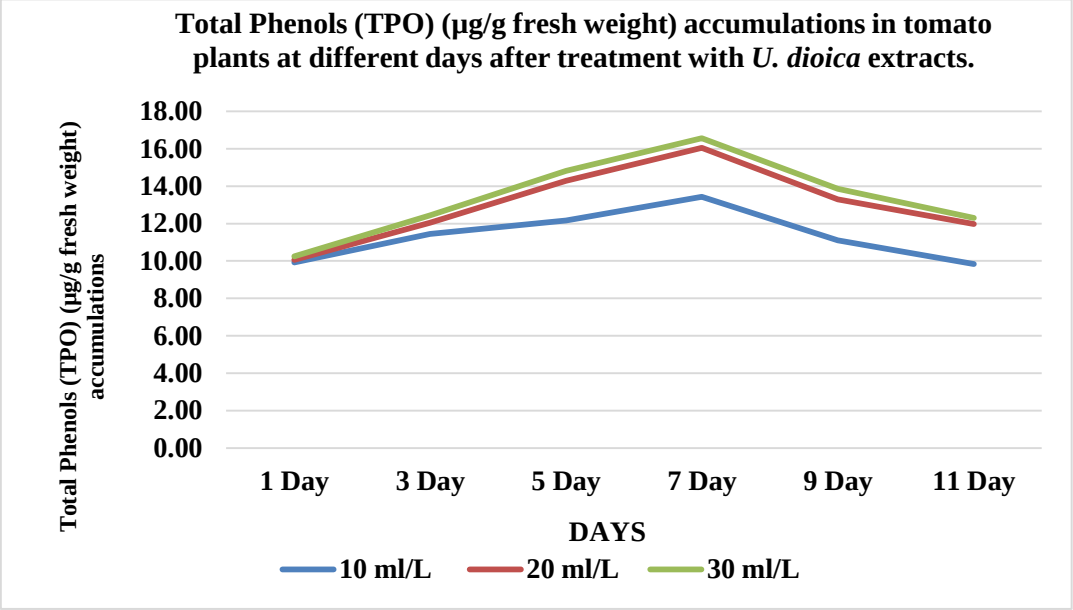


Figure 7-Total Phenols (TPO) (µg/g fresh weight) accumulations in tomato plants at different days after treatment with *U. dioica* extracts.

In this work, we evaluated the fungicide activity methanolic and aquatic extracts of two medicinal plants with their Nano-biosynthesized against *B. cinerea* in tomato plants. The AMPB extracts were the most reducing growth of *B. cinerea* on tomato plants. After seven days of treatment, the results showed increasing TPO in tomato leaves. This may be due to the high concentration of total phenolic compounds (Figure-7). The phytochemical compounds from *U. dioica* and *P. nigrum* are lectins, sterols, terpenes, polysaccharides, volatile compounds and fatty acids, vitamins, protein, minerals and flavonoids [19,20]. Also, AgNPs has antibacterial activity both in nanoparticle and ionic forms. The AgNPs effect on the growth was analyzed and revealed effectively inhibit the hyphal growth in a dose-dependent manner. However, Min, *et al*., [21] found severe damage in hyphae of the fungi after exposed to AgNPs, the microscopic observation showed separation of layers of hyphal wall and collapse of fungal hyphae. While IJato, *et al*., [22] suggest that the Ag^{+}, inhibited colony growth of the microbial and showed that Ag^{+} and NPs reduction in disease severity when applied three hours prior to pathogen inoculation.

The treatment with AgNPs instead of commercial ant-fungal has been shown by Lamsal, *et al*., [23]. They study the effect of this NPs on Colletotrichum species related with pepper anthracnose under many culture environments and concluded that the concentration of 100 ppm inhibited the growth of fungal hyphae as well as conidial growth *in vitro* compared to the control treatment. The result of treatment with the AgNPs also showed significantly high inhibition of fungi in field culture when sprayed the plants before disease outbreak [23].

The increasing TPO in infected plants at the infection site has been correlated with the restriction of microbial development, at such compounds became more toxic to pathogens. Also, TPO might impede pathogen pollution by increasing the mechanical strength of the cell wall membrane [24]. This may be because of the initiation of systemic resistance in the infection plant due to treatments. The increase in production of TPO resists the advancement of the microbial against the other healthy cells. The higher TPO after exposure to fungitoxic in nature and their accumulation enhanced the mechanical strength to the cell wall resulting in the inhibition of microbial attack.

Conclusions

According to the results obtained from this study, AMPB may be an alternative, easy to use, safe and cost-effective control method for the chemical control of gray molds from tomato plants in commercial greenhouses. Further studies should be conducted to determine the active compounds responsible for each station's countermeasures and to assess the cost and efficacy of these extracts on a wide range of diseases. This study concludes that inducing the plant's own defense mechanism by applying biocontrol agents can be an effective strategy in plant disease management.

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