ARTICLE / INVESTIGACIÓN

Biological and chemical control of *Ectophoma multirostrata* causing root-rot and seedling death of *Celosia argentea* in Karbala/Iraq

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Abstract: This study was conducted in the College of the Agriculture/University of Karbala to control the fungus *Ectophoma multirostrata* that causes root rot of *Celosia argentea* by using *Azotobacter chrocooccum*, Salicylic acid and the chemical pesticide Beltanol. The pathogenic *E. multirostrata* was isolated for the first time in Iraq and showed a reduction in seed germination by 16.66% and 16.00%. The results showed that the bio-control bacteria *A. chrocooccum*, Salicylic acid and Beltanol effectively reduced the infection rate and severity of Celosia argentea root rot disease and increased the growth parameters. Among the treatments, Beltanol was the highest in reducing the infection rate and severity down to 0.00%, followed by the treatment of integration between *A. chrocooccum* and Salicylic acid to minimize infection and severity to 16.33% and 8.00%, compared to the infected untreated that showed 80%, 62.00% respectively. In addition, the *A. chrocooccum* and Salicylic acid integration improved plant growth, including shoot length, shoot and root dry weight to be 22.50 cm, 0.423 g and 0.133 g, compared to the untreated infected treatment that resulted in 5.00 cm, 0.090 g, and 0.003g, respectively.

Key words: *Celosia argentea*, *Ectophoma multirostrata*, *Azotobacter chrocooccum*, Root rot.

Introduction

Celosia argentea is exposed to several pathogens, such as leaf spot, root and stem rot, blight and root knot nematodes[1]. Root rot and seedling death are among the most important of these diseases, as they pose a significant threat where the damage begins indistinguishable underground. The disease can't be controlled primarily when the symptoms appear on the upper part of the plant, usually combined with advanced stages of injury. Symptoms associated with root rot diseases are the transformation of roots color to brown with soft affected tissues; they become tender and decomposed. The spots on the roots vary in number, size and color - from reddish to brown and black, with root splintering. The infected plants show leaf yellowing, plant stunting and low yield[2,3]. The progression of root disease pathogens depends on the availability of favorable conditions or recurrent and other factors that contribute to plant stress. Many soil-borne pathogens can cause these diseases, some of which are host-specific and others are of a broader range of plant hosts[4]. *Ectophoma multirostrata* was reported as a cause of root rot disease on a limited number of plants and was recorded on chickpeas in India[5]. Because of the importance of this disease and the absence of previous studies in Iraq on root rot disease on *Celosia argentea*, and to reduce the damage caused by chemical pesticides, the research was conducted to evaluate using safe control methods such as bacteria, *Azotobacter chroococcum* and Salicylic acid in comparison to chemical pesticide Beltanol.

Materials and methods

The fungi accompanying the roots of *Celosia argenta* were isolated, which had symptoms of weak growth, yellowing of the vegetative system and rotting of the root system. Infected samples were collected from some plant nurseries located in Karbala Province. The roots were washed well, cut into small pieces (1-1.5 cm), sterilized with sodium hypochlorite solution, transferred to Petri dishes containing more dextrose (PDA) medium, and incubated at 25 ± 2°C for three days. Fungi were purified and initially diagnosed based on phenotypic traits using the taxonomic keys described previously[6-8]. In addition to their molecular diagnosis in a previous study by analyzing the sequences of the nitrogenous bases of the DNA products by PCR for selected genetic markers and using the BLAST (Basic Local Alignment Search Tool) program, these isolates from the roots of *Celosia argentea*, are *Ectophoma multirostrata* (Ec2) and are registered in the NCBI under accession number ON025673[1].

Pathogenicity of fungal isolates on red radish seeds on Water Agar media

The pathogenicity of the three isolates of the fungus was tested (Table 1). The fungi were isolated from the roots of infected *Celosia argenta* plants by using plastic plates[9] and using a water agar medium. The susceptibility of radish seeds to infection with fungal isolates was tested in comparison to the control treatment based on the percentage of germination[2]. As well as calculating the percentage of inhibition following Abbott's (1925)[10] equation[11].

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Pathogenicity of fungal isolates on *Celosia argentea* seeds in plastic pots under greenhouse conditions

This experiment was carried out by mixing soil mixture with peat-moss (1:2) and sterilizing it using an autoclave at a temperature of ˚121°C and a pressure of 15 psi/ing2 for 60 minutes. Then the soil was inoculated with the fungus-bearing millet seeds (*Panicum miliacem*) at a percentage of 1% for 48 hours[12], then planted with *Celosia argenta* seeds at a rate of 10 seeds/pot and watered carefully as needed. The germination rate was calculated 30 days after planting.

Effect of the three experimental factors on root-rot fungus *E. multirostrata*

Antagonistic of *Azotobacter chroococcum* to *E. multirostrata* on PDA culture medium was evaluated. Several dilutions of *A. chroococcum* were used to determine the best concentration against the pathogenic fungus *E. multirostrata*, and the percentage of inhibition was calculated[13]. The efficiency of fungicide Beltanol against *E. multirostrata* in PDA was also evaluated at three concentrations, including the recommended by the manufacturer, 0.5 and 0.75% of the recommended concentration. The percentage of pathogen inhibition was determined. Salicylic acid at 0.5, 1.0, and 1.5 $g.L^{-1}$ was also tested against *E. multirostrata* on PDA culture media and the percentage of inhibition was calculated after seven days of inoculation.

A greenhouse pot experiment

The efficiency of *A. chroococcum*, salicylic acid and Beltanol and their integration was evaluated in controlling *E. multirostrata* on *Celosia argentea* under greenhouse conditions.

The inoculum of the pathogenic *Ectophoma multirostrata* loaded on millet seeds was added to the plastic pots at a rate of 1% / pot and with three replications for each treatment. In contrast, the bacterial biological agent *Azotobacter chroococcum* was added to the pot at a rate of 10 ml/pot. The chemical pesticide Beltanol was added at a concentration of 1 ml/liter in addition to a treatment A comparison in which the seeds were sown without addition and with a number. After 48 hours, the seeds were planted in the soil, while salicylic acid was added ten days after the emergence of seedlings. Nine treatments were implemented as the factors were used single and overlapping with the presence of a comparison treatment. After 60 days of applying the experiment, the infection rate was calculated. The pathological key of 6 degrees (0-5) was used to assess the severity of root rot disease[2,14]. Infection severity was also calculated according to Mckinney equation (1923)[15], following Jaber (2020)[11] and Dkhyl (2021)[2]. At the end of the experiment, the plants of each treatment were uprooted to measure shoot length, fresh weight and dry weight.

Treatments and experimental units were distributed as a Completely Randomized Design (CRD) with three replications as a one-factor experiment. Data analysis, analysis of variance ANOVA, and the Least Significant Difference (LSD) among treatments were performed using the GenStat program, 10th edition.

Results and discussion

Three isolates of *Ectophoma multirostrata* were obtained from plants that showed symptoms of infection (Fig. 1-C), as they were characterized by their formation of olive-brown fungal colonies(Fig.1-B). Microscopic examination showed the presence of a divided mycelium, as well as the presence of single-celled oval conidia (Fig. 1-A) and spirochetal C. The shape is dark in color; these results are consistent with what was found by Chobe *et al.* (2020)[5] and Kashyap *et al.* (2022)[16], who isolated this fungus for the first time in the world and recorded it as a cause of root rot and seedling death of chickpea seedlings.

Testing the pathogenicity of the fungi isolated in this study

Pathogenicity of fungal isolates on red radish seeds on Water Agar media

The results (Table 2 and Figure 2) showed that all tested isolates of mushrooms led to a significant reduction in germination percentage, compared to the comparison treatment in which the seed germination ratio was 100%. The other isolates led to a reduction in the rate of seed germination to 16.66%. The isolates varied among themselves in the decrease in germination percentage, which may be attributed to genetic differences within the same species collected from different regions or differences in their ability to secrete pectin- and cellulose-degrading enzymes in the early stages of infection. Enzymes such as pectinase, phosphatase, cellulase, methyl esterase, and methyl hydrolase are involved in host penetration, which significantly influences the pathogenicity of the fungus, in addition to the ability of these fungi to produce some toxins of phenolic and glycoside nature[2].

Three isolates of *Ectophoma multirostrata* were obtained from plants that showed symptoms of infection (Fig. 1-C), as they were characterized by their formation of olive-brown fungal colonies(Fig.1-B). Microscopic examination showed the presence of a divided mycelium, as well as the pres.

Pathogenicity of fungal isolates on *Celosia argentea* seeds in plastic pots under greenhouse conditions

The results (Table 3) indicated that the addition of isolates of the fungus *Ectophoma multirostrata* led to a reduction in the germination of seeds compared to the comparison treatment, which had a percentage of germination, which was 100%. The seed germination rate is 16.00%, and the percentage of inhibition is 84.00%, followed by a difference in the Significance of isolate Ec1, which reached 20.2% and 79.8%, respectively. Based on these results, and agreeing with the previous studies[17] isolate, Ec2 was chosen for use in subsequent experiments.

Location of collection	Isolate symbol (abb.)	Location of collection
Karbala-Hussainiya	Ec1	Karbala-Hussainiya
Karbala-Alhur	Ec2	Karbala-Alhur
Karbala-Ibrahimiya	Ec3	Karbala-Ibrahimiya

Table 1. Fungal isolates from *Celosia argenta* infected roots and dead seedlings collected from different Karbala areas.

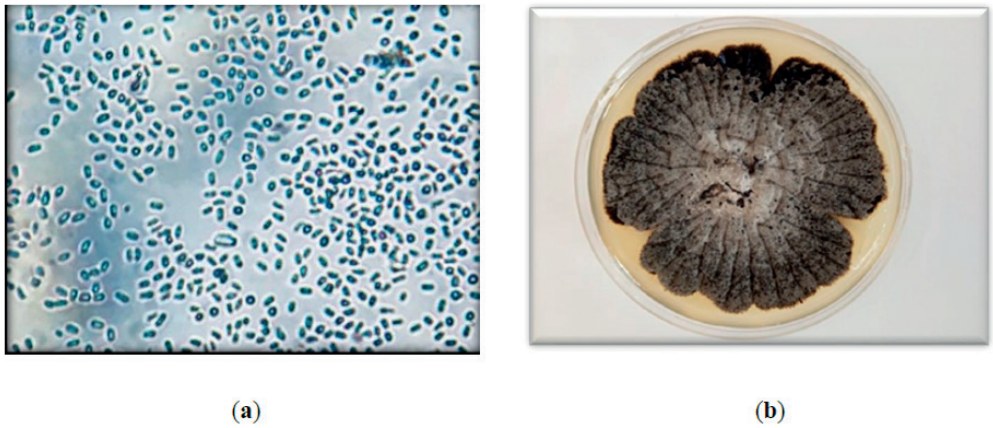

Figure 1. Phenotypic characteristics of Ectophoma multirostrata E2 isolated from *Celsia argentea* plants. (a) A pure culture of *E. multirostrata* on PDA, (b) a Micrograph (40X) of the spores of *Ectophoma multirostrata*, and (c) healthy (right) and infected (left) plants.

Table 2. Detection of pathogenic isolates using red radish seeds on Water Agar.

Isolation symbol	% for germination	% to inhibit
Control	100.0	00.00
Ec1	53.33	46.67
Ec2	16.66	83.34
Ec3	60.00	40.00
LSD 0.05	2.478	2.609

[1] Each number in the table represents an average of three replicates, Ec= *Ectophoma multirostrata*

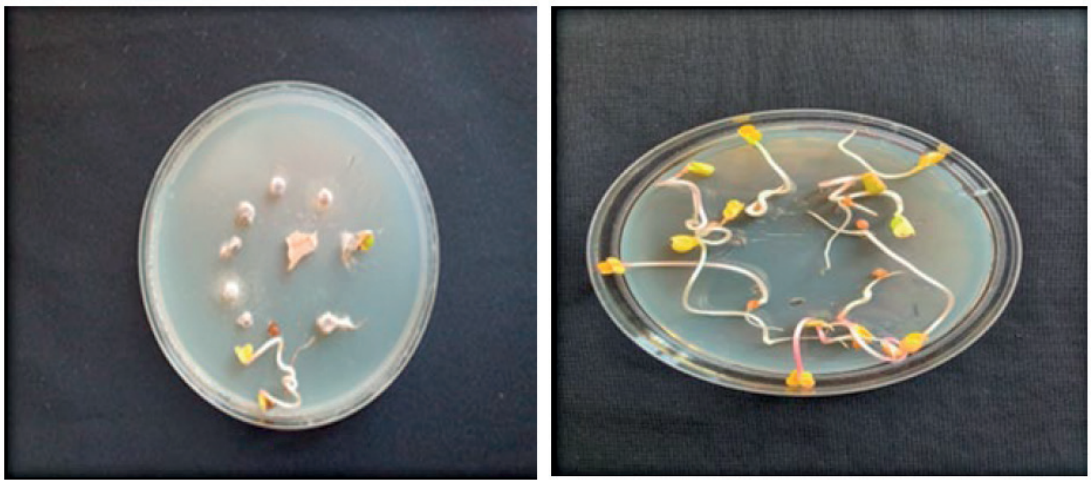

Figure 2. Shows the pathogenicity test using red radish seeds on a Water agar medium (WA). *Ectophoma multirostrata* (Ec2), A=Control*

Isolation symbol	% for germination	% to inhibit
Control	100.0	0.00
Ec1	20.2	79.8
Ec2	16.00	84.00
Ec3	22.6	77.4
L.S.D. (P≤0.05)	1.3055	1.3055

[1]Values are means of three replicates, Ec= *Ectophoma multirostrata.*

Table 3. Pathogenicity of *Ectophoma multirostrata* isolates on seed germination of *Celosia argentea* in plastic pots under greenhouse conditions.

Control of the fungus *Ectophoma multirostrata*, the causes of root rot and damping off on *Celosia argenta*

Antagonistic ability of *Azotobacter chroococcum* against *Ectophoma multirostrata*

The results (Table 4 and Figure 3) showed the ability of *A.chroococcum* bacteria to inhibit the growth of the pathogenic fungi *Ectophama multirostrata* (Ec2) isolated in this study on PDA culture media. % compared with the treatment of pathogenic fungi alone, which amounted to 0.00%. The results showed that there is a direct proportion to the percentage of inhibition by increasing the concentration of bacteria, as it caused a significant reduction in the growth of isolate Ec2. The higher the concentration of *A. chroococcum* bacteria, the higher the inhibition percentage compared to the fungus treatment alone, which amounted to 0.00%. herbicolin[18]. In addition, *A. chroococcum* can produce low molecular weight compounds that function to resist pathogenic fungi, including hydrogen cyanide (HCN). The presence of this compound in high concentrations inhibits the growth of pathogenic fungi[19], and it has a strong ability to compete with pathogens for iron through its production of siderophores[20]. The production of many compounds useful for plant growth, such as ammonia, vitamins and growth regulators such as indole acetic acid, gibberellin and cytokinin, promote seed germination and plant growth[21,22].

These results agree with the results of other studies that found the inhibitory ability of *A. chroococcum* bacteria for many plant pathogens. It showed its high antagonistic ability when used directly or the filtrate to inhibit the growth of the fungi *R. solani* and *F. solani* that cause the death of tomato seedlings[19]. It has also been shown to inhibit *Marcelleina persoonia, Fusarium oxysporum, fungi Lasiodiplodia theobromae, Fusarium equiseti, Curvularia lunata , Cochliobolus, Trichocladium griseum* Causes root rot and damping off several ornamental plants on PDA culture media2.

The results showed (Figure 4) that the chemical pesticide Beltanol. It led to the inhibition of the fungus *Ectophoma multirostrata* (Ec2) by 100% using the concentrations recommended by the producing company and 0.5 and 0.75% of the recommended concentration. These concentrations did not differ significantly among themselves in the percentage of inhibition of pathogenic fungi, which amounted to 100%. The effect of the chemical pesticide Beltanol on pathogenic fungi may be attributed to its ability to form chelating compounds with copper inside its host tissues, thus facilitating the process of its passage into the pathogen's cells and then liberating and killing the pathogen[2,23]. Such effects of the pesticide may also be due to the active substance (8-Hydroxyquinoline), which is known for its effectiveness against a wide range of plant pathogenic fungi. And the effect of this substance on fungi is due to causing ab-

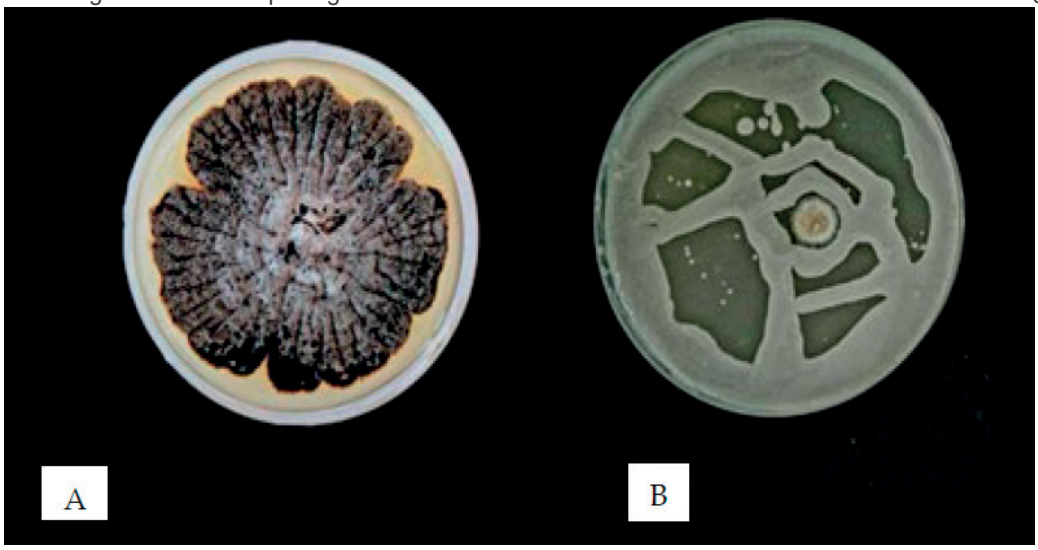

Figure 3. The antagonistic ability of the biological agent *A. chroococcum* against the fungus *E. multirostrata* (Ec2), A=Control B= *Ectophoma multirostrata*+ *A.chroococcum*.

Treatment	Dilution	Average Fungi colony diameter (cm)	% to inhibit
Ec2+Azo	100	9.00	0.00
	10-1	0.00	100.00
	10-2	0.00	100.00
	10-3	0.00	100.00
	10-4	0.50	94.44
	10-5	1.00	88.88
	10-6	3.00	66.66
	10-7	5.00	44.44
	10-8	6.50	27.77
LSD. (P≤0.05)		0.338	4.5593

[1] Values are means of three replicates, Ec= Ectophoma multirostrata

Table 4. The antagonistic ability of *A.chroococcum* against the fungi that cause root rot and damping off *Celosia argentea* on PDA culture medium.

normalities in fungal cells, changing the permeability of cell membranes, leaking their contents to the outside, and inhibiting the formation and germination of Sclerotia bodies[24,25].

Evaluation of the efficacy of salicylic acid against fungi causing root rot and damping off *Celosia argentea* in PDA culture medium

The results showed (Table 5 and Figure 5) the effectiveness of all used concentrations of salicylic acid in inhibiting the growth of the fungus *Ectophoma multirostrata* (E2). The comparison treatment, in which the diagonal growth rate was 9.0 cm and the concentration of 1.5 g / liter, significantly exceeded the other concentrations of *Ectophama multirostrata* (Ec2) inhibitor 2.5 cm, with a growth inhibition rate of 72.2%. The concentration of 0.5 gave the slightest effect in inhibiting the pathogenic fungus. Still, it differed significantly from the control treatment and reached a growth rate of the fungus at 6.2 cm and an inhibition rate of 33.11%. The results show a positive relationship between the increase in acid concentration and the increase in the percentage of inhibition. The effectiveness of salicylic acid may be attributed to its inhibition of many vital processes in pathogenic fungi, such as the action of enzymes and amino acids, and then affecting the activity and growth of pathogens[26,27]. These results agree with the findings of Hassan (2005)[28] indicating complete growth inhibition of *Pythium aphanidermatum* on PSA culture media when SA was used at 400 ppm. Similarly, a direct relationship was found between the concentration of salicylic acid and the percentage of growth inhibition of the fungus *Pythium aphanidermatum* that causes seed rot disease and cucumber death[29].

Effect of *A. chroococcum*, salicylic acid and Beltanol in controlling *E. multirostrata* on *Celosia argentea* under greenhouse conditions

The results showed (Table 6) that all the factors used effectively reduced the percentage of infection and its severity and increased growth parameters compared to the infected untreated control. Beltane fungicide was superior in reducing the affection rate and its seriousness to 0.00%, followed by Az+Sal+Ec2 treatment, which amounted to 16.33%, and 8.00%, respectively. Shoot length and dry wei-

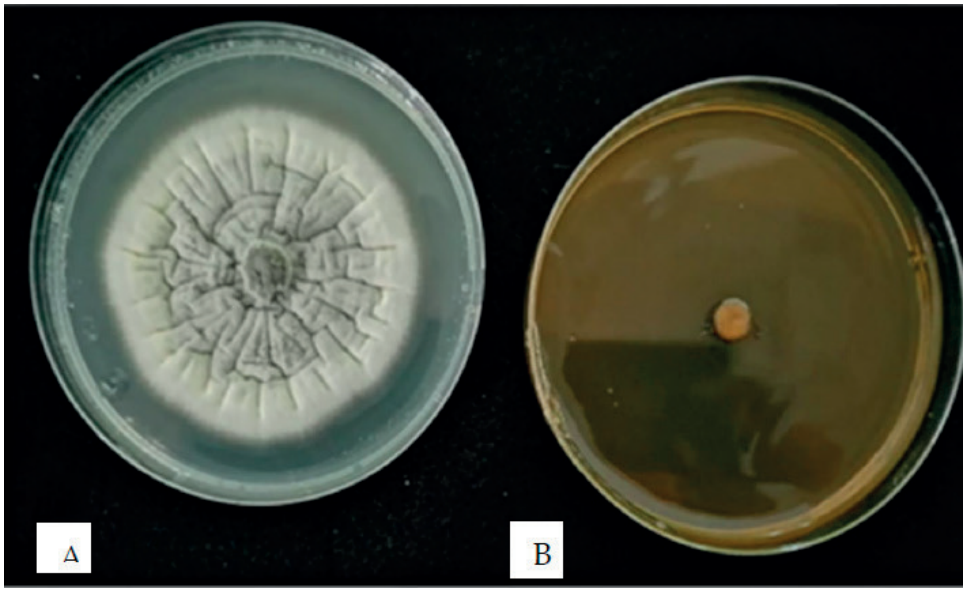

Figure 4. Antagonistic ability of the chemical pesticide Beltanol against the fungus that causes root rot and seedling death of *Celosia argentea* on PDA medium. A= *E. multirostrata* (control) and B= *E. multirostrata*+Beltanol.

Concentration g/L	Average Fungi colony diameter (cm)	% inhibition
Control	90.0	0.00
0.5	6.2	3.11
1	3.5	61.1
1.5	2.5	72.2
LSD. (P≤0.05)	0.8804	0.5111

[1]Values are means of three replicates, Ec= *Ectophoma multirostrata*.

Table 5. Salicylic acid effect on *E. multirostrata* growth on PDA culture media

Figure 5. Salicylic acid (SA) Efficiency against the pathogenic *Ectophoma multirostrata* in PDA culture medium. A= *E. multirostrata* (control), B= *E. multirostrata*+SA

ght of vegetative and root groups 22.50 cm, 0.423 g, and 0.133 g, respectively, as well as all factors used without the presence of the causes to increase growth parameters[2,23]. The pesticide is converted to the active substance (8-Hydroxyquinoline), which is known for its effectiveness against many plant pathogenic fungi. One of the substances derived from this active substance proved its inhibitory efficiency against the fungi *Sclerotinia sclerotiorum, Fusarium graminearum, Magnaporthe oryzae* and *Ilyonectria liriodendra*. And it was inhibiting the formation and germination of Sclerotia bodies[24,25].

The effectiveness of salicylic acid may be due to its inhibition of many vital processes in pathogenic fungi, such as the action of enzymes and amino acids, and then affecting the activity and growth of these pathogens[26,27].

Conclusions

The study showed the presence of the fungus *Ectophoma multirostrata* accompanying the symptoms of root rot on the *Celosia argentia*. This plant host recorded the pathogenic fungus for the first time in Iraq. The study also showed the possibility of reducing infection by using *Azotobacter chrocooccum*, salicylic acid and the chemical pesticide Beltanol. In general, the chemical pesticide had the highest effect in reducing the rate and severity of infection, followed by the combination treatment of *A. chrocooccum* and salicylic acid. The contrast of fungal isolates, in their ability to sicken and their inhibition of *Celosia argentea* seed germination, may be attributed to the genetic difference between fungal isolates of the same species that were collected from different regions or due to the difference of isolates in their ability to secrete pectin- and cellulose-degrading enzymes in the early stages of infection. These enzymes play a role In penetrating the family, and from it, Pectinase, Phosphatase, Cellulase, Methylesterase, Pectinmethylhydrase, and Protase, which have a significant effect on the pathogenicity of fungi, as well as the ability of these fungi to produce some toxins of a phenolic and glycoside nature

The effect of *A. chroococcum* bacteria may be attributed to its high ability to produce some antifungal compounds, metabolites, organic compounds, and several enzymes that can degrade the cell walls of pathogenic fungi, including

Treatment	Infestation rate (%)	Infection severity (%)	Plant height/ cm	Shoot Dry weight (g)	Root Dry weight (g)
Control	0.00	0.00	21.00	1.32	0.012
Ec2	80.00	62.00	5.00	0.090	0.003
Az	0.00	0.00	24.00	2.03	0.051
Sal	0.00	0.00	24.00	2.02	0.051
Bel	0.00	0.00	22.00	2.10	0.041
Az+Sal	0.00	0.00	34.00	2.45	0.693
Az+Ec2	45.33	37.66	16.50	1.09	0.067
Sal+Ec2	46.00	38.00	16.33	1.09	0.067
Bel+Ec2	0.00	0.00	21.83	2.02	0.312
Az+Sal+Ec2	16.33	8.00	22.50	0.423	0.133
LSD0.05	0.6189	0.5054	0.8047	0.0059	0.0949

[1] Values are means of three replicates, Sal=Salicylic acid, Bel=Beltanol, Ec=*E. multirostrata*, Az=*A. chroococcum*.

Table 6. Effect of *A. chroococcum*, salicylic acid and Beltanol and on pathogenicity of *E. multirostrata* on *Celosia argentea* under greenhouse conditions

Glucanase, Chitinase, laminarinase, and the production of several antibiotics such as Phenazine, Pyoluteorin.

The effect of the chemical pesticide Beltanol on pathogenic fungi may be attributed to its ability to form chelating compounds with copper within its host tissues, thus facilitating the process of its passage into the pathogen's cells and then liberating and killing the pathogen. The same combination also led to a clear improvement in the studied indicators of plant growth.

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