Salahaddin University -Erbil

College of Agricultural Engineering Sciences

Department of Plant Protection

Stage: Fourth stage

Subject: Research

Title of the Scientific Report

In vitro control of *Alternaria solani* the causal agent of potato early blight by plant extratcs.

First Semester

2022-2023

Subject's In-charge

Mr. Mohammad zrar &Mrs. Nask Salh

Student's Name:

Mohammad Fadel Mohammad

Abstract:

Early blight is distructive disease of potato caused by Alternaria solani. To inhibit the linear growth of the causal agent two plant extracts Nerium oleander Allium sativum were used invitro, both of plant extracts showed best result in reducing linear growth by 85.26 and 81.41respectively.

1. Introduction:

The potato (*Solanum tuberosum L*) is a starchy tuber of the plant belongs to the nightshade family Solanaceae and is a root vegetable native to the Americas (Hijmans, 2001).

Potato (*Solanum tuberosum L*.) is the fourth major agricultural crop after corn, wheat and rice, and it is the first major vegetable among vegetables called (king of vegetables) which is used in many countries as the main foodstuff (Lovat, *et al*, 2016 and Zhang *et al*, 2017). In 2019, the global potato production in the world reached 370.5 million tons (Soare and Chiurciu, 2012-2019).

Potato is excellent source of vitamins, amino acids, carbohydrate,proteins, minerals and phytochemicals such as phenolic compound and carotenoids2. Presence of these nutrients reduces risk of several diseases such as cancer, diabetes, cataract and aging (Nunn and Qian, 2011).

Widespread viral, fungal, and bacterial diseases like Common Scab (Streptomyces spp.), Fusarium Dry Rot (*Fusarium spp*.), Black Dot (*Colletotrichum coccodes*), Black Scurf and Rhizoctonia Canker (*Rhizoctonia solani*), Potato Early blight (*Alternaria solani*) are the reasons for poor potato yield is which infect plants and are stored in the tubers. In many cases, chemical changes in the infected tubers make them unsuitable for processing. Along with the late blight of potato, early blight of potato represents one of the most harmful and common potato diseases causing significant reduction in the tuber yield and quality (Kokaeva et al, 2018, Zhang *et al*, 2020). According to some data, poor control of this disease may result in yield losses reaching 50–58% (Metz and Hausladen, 2022)

Early blight is caused by necrotrophic imperfect fungi from the genus Alternaria solani (Gorai *et al*, 2021). The primary infection sources include conidia, chlamydospores, and mycelial debris overwintered in soil or plant debris (Van der Waals et al, 2001). Alternaria fungi mainly infect plants weakened by viral diseases or suffering from the deficiency of mineral nutrition.

1.3 Aims of study:

1. To determine the efficacy of plant extracts on the *Alternaria solani*.

2- Choosing the best plant extracts for inhibition the growth of Alternaria solani

2. Literature Review:

Early blight of potato is caused by the fungal pathogen Alternaria solani. The disease affects leaves, stems, and tubers, and can reduce yield, tuber size, storability of tubers, quality of fresh-market and processing tubers, and marketability of the crop (Bauske *et al*, 2020).

2.1 SYMPTOMS:

Foliar symptoms of early blight appear as small, irregular to circular dark brown spots on the lower leaves. These spots may range from pinpoint to 1/8 inch in diameter.as the spots enlarge they become restricted by leaf veins and take on an angular shape.

Figure 1. Early blight lesions initially appear as small, circular to irregular dark-brown spots on older (lower) leaves.https://www.ndsu.edu/agriculture/ag-hub/publications/early-blight-potato.

Leaf lesions are relatively easy to identify in the field because lesion development is characterized by serious of dark concentric rings alternating with bands of light tan tissue (figure 2). (Bauske *et al*, 2020).

Figure 2. Initial early blight lesions on older leaf tissue https://www.ndsu.edu/agriculture/ag-hub/publications/early-blight-potato

By the end of the growing season the upper leaves of infected potato plants may be peppered with numerous small early blight lesions and subsequently, lesions may coalesce cover a large area of the leaf (Bauske *et al*, 2020) (figure).

Figure 3. Multiple early blight lesions on the same leaf may coalesce, or grow together https://www.ndsu.edu/agriculture/ag-hub/publications/early-blight-potato

Premature leaf senescence, reduced yield, and low dry matter content likely will result from severe foliar infection during the tuber bulking stage, also Severely infected leaves eventually wither and die but usually remain attached to the plant (figure) (Bauske *et al*, 2020).

Figure 6. As early blight progresses during the season, numerous lesions may appear on younger leaves in the upper canopy and leaves may drop or dehisce from the stem as infection becomes severe https://www.ndsu.edu/agriculture/ag-hub/publications/early-blight-potato

Symptoms of early blight infection on tubers appear as dark and sunken lesions on the surface (Figure 7). Tuber lesions may be circular or irregular in shape (Figure 8) and can be surrounded by a raised dark-brown border (Bauske *et al*, 2020).

Figure 7. Early blight symptoms on infected tubers appear as dark-colored, sunken lesions on the tuber surface

Figure 8. Early blight tuber lesions may be circular or irregular in shape and often are accompanied by a large, raised dark-brown border

https://www.ndsu.edu/agriculture/ag-hub/publications/early-blight-potato

2.2 DISEASE CYCLE:

The primary infection of potato foliage by *A. solani* is caused by inoculum provided from other infected hosts or inoculum that overseasoned on infected plant debris. Overwintering spores that serve as the initial inoculum move within and between fields carried by air currents, windblown soil particles, splashing rain, and irrigation water (Harrison *et al*, 1965).

Following initial infection, sporulation occurs on lesions, and spores are dislodged under conducive environmental conditions (Figure 9). Alternating wet and dry periods are most favorable for sporulation and dispersal. The spores produced by primary inoculum are responsible for secondary spread of the fungus to healthy

tissue, which leads to an exponential increase of foliar infection (Douglas and Groskopp, 1974).

Figure 9. Spores of *Alternaria solani* (https://www.ndsu.edu/agriculture/ag-hub/publications/early-blight-potato)

Minimum and maximum temperatures for sporulation and infection of potato by *A. solani* are 41 and 86 F, respectively, with an optimum temperature of 68 F. Following sporulation, spores are disseminated by wind or splashing from rain or irrigation water, and the number of airborne spores peaks in midmorning and declines throughout the afternoon and at night (Escuredo *et al*, 2019).

Under high humidity and free moisture, and within the range of temperatures, spores landing on leaves of susceptible plants germinate and may penetrate host epidermal cells directly or enter through stromata or wounds. Many cycles of early blight spore production and lesion formation occur within a single growing season.

In addition to survival on infested plant debris, spores and mycelia of *A. solani* can survive between growing seasons in infected potato tubers and in the overwintering debris of other susceptible solanaceous crops and weeds, including tomatoes and hairy nightshade. Additionally, spores can survive freezing temperatures on or just below the soil surface (Da Silva *et al*, 2021).

During harvest, tubers often are contaminated with *A. solani* spores that accumulated on the soil surface during the growing season or were dislodged from desiccated vines. Germinated spores penetrate the tuber epidermis through lenticels and through wounds on the tuber surface caused by mechanical injury (Workman and Harrison, 1980).

2.3 antifungal activity of Plant extract in controlling *Alternaria solani*:

Potato production is constrained by fungal diseases especially the early and late blight caused by *Alternaria solani*. Control of the disease is usually by use of synthetic fungicides which have a long residue effect and also contribute to environmental pollution. Innovative use of biocontrols may offer an eco-friendly and more sustainable solution. A study were tested 27 plant extracts against *Alternaria solani* by uaing radial growth and the result revealed that only 4 plant extracts showed significant antifungal activities and the effect of these four plant extracts in inhibiting the mycelial growth of *Alternaria solani* were *Lauris nobilis* (79.35%) at 4% concentration, *Salvia officinalis* (76.50%), *Humulus lupulus* (61.50%) and *Circium arvense* (55.83%) (Yanar *et al* 2011).
And another report six plant extracts were tested against *Alternaria solani* and only *Allium sativum* at 1% concentration showed 86.2% inhibition, 94.4% inhibition at 2% concentration, and 100% inhibition was achieved at 3% and 4% concentrations (Olivia *et al* 2017).
Also in a study evaluated the effect of two plant extracts, Ricinus communis and Chromolaena odorata on the control of the early blight pathogen, *Alternaria solani* by three concentration (25%, 50%, and 100%) and the result revealed that Ricinus communis at 100 % concentration was recorded for the lowest radial growth rates of 1.43 cm, 2.00 cm and 2.72 cm at 24, 48 and 72 hours were recorded, respectively (Nahunnaro and Bayaso, 2012).

3. Materials and methods:

3.1 Sample collection:

Infected potato leaves which showed early blight symptoms were collected and taken to plant pathology laboratory for isolating causal agent and control of isolated fungi by plant extracts (*Alium sativum* and *Nerium oleander*)

3.2 Isolation of the fungus:

The infected leaves were cut into small pieces and the pieces were surface sterilized by sodium hypochloeide and then washed by distilled water, after that the small pieces were placed on the PDA media and taken in the incubator they were left for about a week.

3.3 In vitro control of *Alternaria solani* by plant extracts:

In this experiment 10g of each plant extracts were taken in 100ml of water. These were left for 24 h and subsequently filtered through Buchner funnel. Then the filtrates were used as plant extract and directly mixed with PDA 1:4 then poured into plates with 4 replications were used and negative control contain only PDA. Then the plates were inoculated with pathogenic fungus. The inoculated plates were incubated at 25°C for about 1 week (Gwa *et al* 2017). The percent growth inhibitions were measured according to the following formula (Gwa *et al* 2017).

$$\text{Percent growth inhibition (PGI)} = \frac{R-R1}{R} * 100$$

PGI=Percent Growth Inhibition.
R=the distance (measured in mm) from the point of inoculation to the colony margin in control plate,
R1=the distance of fungal growth from the point of inoculation to the colony margin in treated plate.

4-RESULT

4.1. Isolation and Identification of the fungus

After incubation period the isolated fungus was identified under light microscope which it is *Alternaria solani*.

3.4 4.2. In vitro control of *Alternaria solani* by plant extracts:

In this experiment which two plant extracts were used to inhibit the growth of *Alternaria solani* in vitro, this two plant extracts showed the best result , the highest inhibition growth rate was recorded from *Nerium oleander* by 85.26% also *Allium sativum* resulted 81.41 % inhibition growth rate as shown in figure (9and 10).

Our result are similar with Kumar et al. (2021) they were tested Allium staivum to reduce the linear growth of Alternaria solani in vitro the causal agent of potato early blight and A. sativum showed significant inhibition of mycelial growth of A. solani by 88.80.

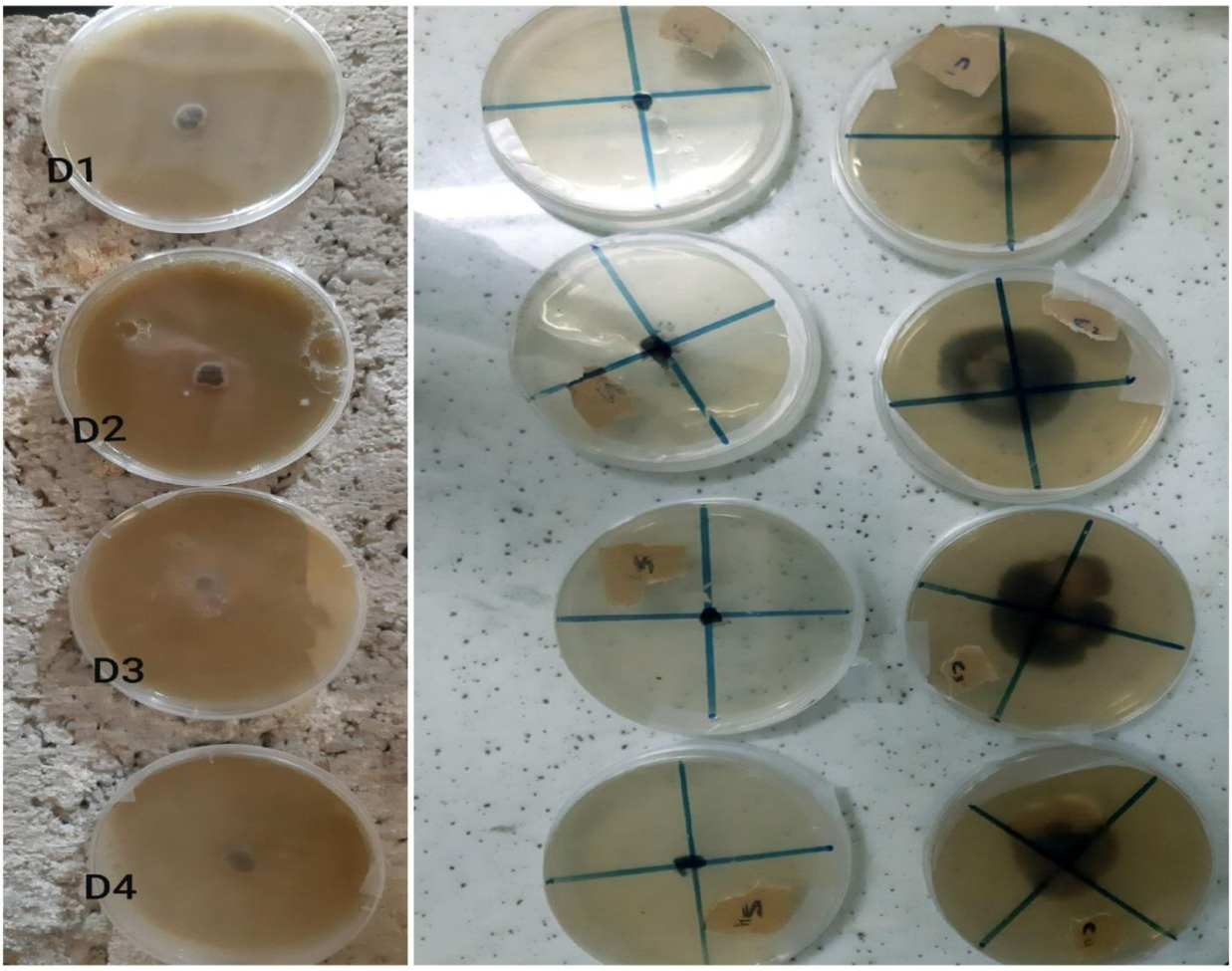

Figure (9): control of *Alternaria solani* by plant extracts were d=*Nerium oleander*, s= *Allium sativum* and c= control.

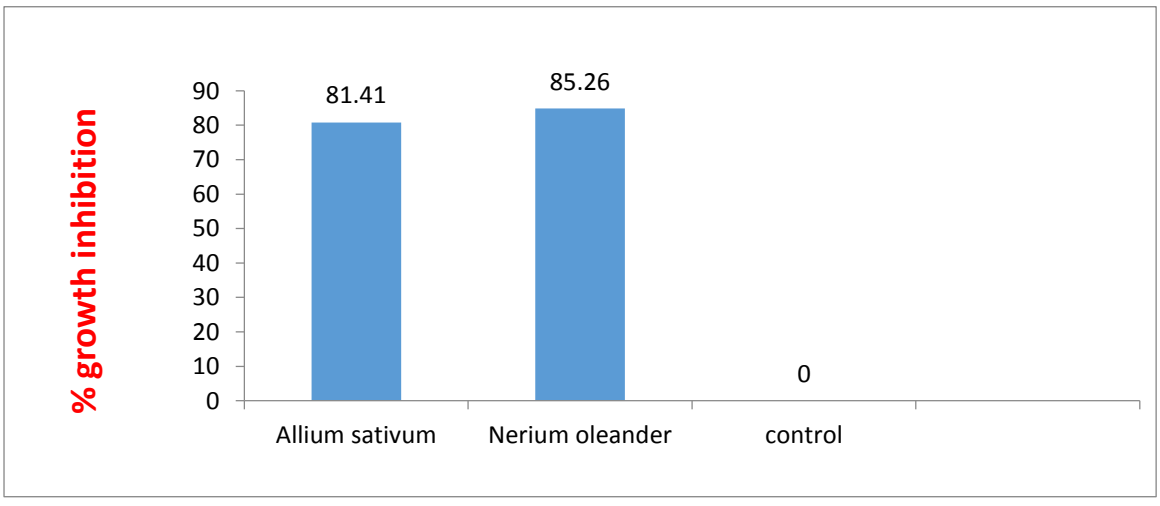

Figure 10. Control of *Alternaria solani* by plant extracts in vitro.

Conclusion

In our research which two plant extracts were used to inhibit the growth of Alternaria solania, both plant extracts showed best result in reducing linear growth of causal agent of potato early blight.

4. References:

1. Bauske, M. J., Robinson, A. P., & Gudmestad, N. C. (2020). Early blight in potato.
2. Da Silva, H.A.O., Teixeira, W.D., Borges, Á.V., Silva Junior, A.L., Alves, K.S., Rodrigues Junior, O.M. and de Abreu, L.M., 2021. Biocontrol of potato early blight and suppression of Alternaria grandis sporulation by Clonostachys spp. Plant Pathology, 70(7), pp.1677-1685.
3. Devi, N. O., Singh, N. I., Devi, R. T., & Chanu, W. T. (2017). In vitro evaluation of Alternaria solani (Ellis and Mart.) Jones and grout causing fruit rot of tomato by plant extracts and bio-control agents. *International Journal of Current Microbiology and Applied Sciences*, *6*(11), 652-661.
4. Douglas, D.R. and Groskopp, M.D., 1974. Control of early blight in eastern and southcentral Idaho. American Potato Journal, 51(11), pp.361-368.
5. Escuredo, O., Seijo-Rodríguez, A., Meno, L., Rodríguez-Flores, M.S. and Seijo, M.C., 2019. Seasonal dynamics of Alternaria during the potato growing cycle and the influence of weather on the early blight disease in north-west Spain. American Journal of Potato Research, 96(6), pp.532-540.
6. Gorai, P.S.; Ghosh, R.; Konra, S.; Mandal, N.C. Biological control of early blight disease of potato caused by Alternaria alternate EBP3 by an endophytic bacterial strain Bacillus velezensis SEB1. Biol. Control 2021, 156, 104551. [CrossRef]
7. Gwa, V.I. and Nwankiti, A.O., 2017. Efficacy of some plant extracts in in vitro control of Colletotrichum Species, causal agent of yam (Dioscorea rotundata Poir) tuber rot. Asian Journal of Plant Science and Research, 7(2), pp.8-16.
8. Harrison, M.D., Livingston, C.H. and Oshima, N., 1965. Epidemiology of potato early blight in Colorado 1. Initial infection, disease development and the influence of environmental factors. American Potato Journal, 42(10), pp.279-291.
9. Hijmans, R.J., 2001. Global distribution of the potato crop. American journal of potato research, 78(6), pp.403-412.
10. Kokaeva, L.Y.; Belosokhov, A.F.; Doeva, L.Y.; Skolotneva, E.S.; Elansky, S.N. Distribution of Alternaria species on blighted potato and tomato leaves in Russia. J. Plant Dis. Prot. 2018, 125, 205–212. [CrossRef]
11. Kumar, S., Chandra, R., & Behera, L. (2021). Assessment of Plant Extracts and their In vitro Efficacy against Potato Early Blight Incited by Alternaria solani. *Journal of Pure and Applied Microbiology*, *15*(3), 1591-1602.
12. Lovat, C.; Nassar, A.M.K.; Kubow, S.; Li, X.-Q.; Donnelly, D.J. Metabolic biosynthesis of potato (Solanum tuberosum L.) antioxidants and implications for human health. Crit. Rev. Food Sci. Nutr. 2016, 56, 2278–2303. [CrossRef] [PubMed].
13. Metz, N.; Hausladen, H. Trichoderma spp. as potential biological control agent against Alternaria solani in potato. Biol. Control 2022, 166, 104820. [CrossRef].
14. Nahunnaro, H., & Bayaso, I. (2012). Inhibitory activity of plant extracts on the early blight pathogen Alternaria solani. *Global Journal of Agricultural Sciences*, *11*(1), 57-62.
15. Nunn N, Qian N. The potato's contribution to population and urbanization: evidence from a historical experiment. Q J Econ. 2011;126(2):593-650. doi: 10.1093/qje/qjr009

16. Soare, E.; Chiurciu, I.-A. Study on the dynamics of potato production and worldwide trading the period 2012–2019. Sci. Pap. Ser. Manag. Econom. Eng. Agric. Rural Dev. 2021, 21, 527–532.
17. Van der Waals, J. E., Korsten, L., & Aveling, T. A. S. (2001). A review of early blight of potato. African Plant Protection, 7(2), 91-102.
18. Workman, M. and Harrison, M.D., 1980. The influence of harvest date on yield, early-blight tuber infection and chipping characteristics of potatoes grown with sprinkler irrigation. General Series, Experiment Station, Colorado State University, (989).
19. Yanar, Y., Gökçe, A., Kadioglu, I., Çam, H., & Whalon, M. (2011). IIn vitro antifungal evaluation of various plant extracts against early blight disease (Alternaria solani) of potato. *African Journal of Biotechnology*, *10*(42), 8291-8295.
20. Zhang, H.; Xu, F.; Wu, Y.; Hu, H.-H.; Dai, X.-F. Progress of potato staple food research and industry development in China. J. Integr. Agric. 2017, 16, 2924–2932. [CrossRef]
21. Zhang, D.; Yu, S.; Yang, Y.; Zhang, J.; Zhao, D.; Pan, Y.; Fan, S.; Yang, Z.; Zhu, J. Antifungal effects of volatiles produced by Bacillus subtilis against Alternaria solani in potato. Front. Microbiol. 2020, 11, 1196. [CrossRef] [PubMed].