University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 2 (2024) PP 217-223

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Effect of Pallas herbicide residue on stimulating the pathogenicity of *Alternaria alternata* in causing leaf spot in cowpea plant

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Abstract

This study was carried out to spotlight on the effect of Pallas herbicide residues of isolates of *Alternaria alternata* in increasing their pathogenicity and stimulating them to cause symptoms of cowpea leaf spot in deferent region of Babylon Governorate, Iraq.

The results of pathogenicity test using radish seeds revealed the ability of three isolates of *Alternaria alternata* to reduce the percentage of radish seeds germination significantly compared with control treatment.

The results showed that the development of the inoculum for the tested isolates on a nutrient medium that was previously prepared with 5 ppm of the herbicide Pallas has contributed to increasing the pathogenicity of these isolates by significantly reducing the percentage of cowpea seeds by following the blotting paper folds technique, Similar results were obtained after supplementation the liquid medium of fungal isolates with 5 ppm of the herbicide Pallas in studying liquid fungal infiltrates in cowpea seedlings infection.

The results of the greenhouse were confirmed with laboratory experiments results, The concentration of 5 ppm of the herbicide Pallas had a significant effect on increasing the pathogenicity of isolates of the fungus *Alternaria alternata*. These pathogens significantly reduced the percentage of germination of cowpea seeds in the potting soil, and reduced the heights of infected plants and significantly reduced dry weight compared to the control treatment. Increasing the appearance of leaf spot symptoms on true leaves of cowpea plants treated with sporophyte suspension prepared with 5 ppm of Pallas herbicide.

Keywords: Alternaria alternate, cowpea, Pallas, herbicide

I. Introduction

Using of herbicides has been a common issue for a long time, and even the modern agricultural method, such as conservation agriculture, relies heavily on the use of herbicides on a large scale. Weeds are often causes an important interference in the agricultural activities of all crops (Devi,et al . 2011) as it competes with crops for area, water and food, and it also causes a significant reduction in the economic value of grain crops when the seeds of these weeds pollute the seeds and grains of important cereal crops (wheat, barley, rice and corn) Khatam, et al (2012), in addition to the fact that many weeds have toxic effects for humans and livestock (Leo and Nollet. 2004).

Recently, the Pallas Iraq herbicide was introduced and its use spread widely in wheat crops(AL-hailly and Abudulkader,2014), and it had an effective effect in successfully controlling weeds (Anonymous, 2010).

Many studies and publications indicate that there are direct or indirect effects of herbicides on pathogens, especially fungi. The use of herbicides often stimulates the activity of pathogens of fungi. This

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phenomenon was first observed during the 1940s(smith. At el. 1946) . Then there were studies on this phenomenon (Simth and Hallett. 2006; Trebst, 2008).

The fungus *Alternaria alternata* (Fries) is a common pathogen spread worldwide and may sometimes cause severe diseases of leaf spot, The leaf spot disease caused by *A. alternata* was observed in all cultivated areas in 1986. This disease and charcoal rot, (*Macrophomina phaseolina* (Tassi) Goid), were the major diseases on the crop (31). Symptoms began as brown zonate spots on basal leaves and progressed to upper leaves. Spots eventually coalesced, and the infected leaves dried up and defoliated (Lagopodi and Thanassoulopoulos. 1996). Severity of infections varied from premature declining of lower leaves to extensive defoliation of entire plants (Berig, et. Al. 2013)

Cwopea plants *Vigna unguiculata* is one of the most consumed plants of the leguminous family in Iraq. Its cultivation spreads in the summer desert in most of the central region of Iraq. It is characterized by its low need of water, its tolerance of hot weather, its rapid growth and good grain production. It is infected with many pathogens, especially leaf spot (Abboud and Al-Jassani. 2021).

This study was amid to evaluate the unsuspected action of Pyroxsula compound (Pallas OD 45) herbicide specially in low concentration to stimulating pathogenicity of *A. alternate* isolates pathogenicity in cowpea plants.

II. Materials and methods

Samples collection:

The process of isolating pathogenic fungi was carried out through field inspection to fields of cucumber ,cowpea and okra which show symptoms of spotting in the autumn grove of Babylon Governorate for the agricultural season 2020 . Four areas famous for the cultivation of these crops were selected in the governorate if 3 random samples were taken from the affected plant leaves from the center ,ALHashimiya ,AL-Kifl ,and two samples from AL-Musayyab area .For the purpose of conducting subsequent studies.

2. Isolation of pathogenic fungi:

A group of infected leaves were taken from each sample superficially sterilized with sodium hypochloride solution (1%free chlorine) for 2 minutes ,washed with sterile water three times ,then dried with sterile filter paper and distributed in the middle of the prepared agar of potato extract and sucrose in petri dishes . 9cm in diameter and 4-5 pieces of 1 thickness in each dish . The dishes were incubated at a temperature of 25 + 2 C^o in the incubator for 7 days ,then all seed samples were examined using a compound microscope ,and the fungi were diagnosed in the Department of Biotechnology using the following keys (Hoching ,pitt) 1977 ,Booth 1997 ,2,,1981 .Domsch (1971 ,Ellis).

3 . Pathogenicity test:

Testing the pathogenicity of the isolated fungi by using the seeds of Radish seeds (*Raphenus sativium*),The experiment included three isolates of the fungus *Alternaria alternata* according to (Butler and Bolken ,1974) by inoculating four petri plates with 0.5 cm discs taken from the edges of each tasted *A. alternata* isolates, after three days ,the seeds were sown after surface sterilization with 1% sodium hypochlorite solution for a period of 2 minutes and washed with sterile water three times, each treatment was forth replicate, all treatments were incubation at 20+2 C . The results were taken after seven days of Radish seeds by taking the percentage of germination and comparing it with the control treatment (Radish seeds in a dish containing water agar without inoculation with any fungus). according to a complete randomized design (CRD), the statistical analysis- Anova table of significant differences were calculated with L.S.D ($\boldsymbol{P} \geq$ o.o5).

4. **Effect (Pallas) herbicide on the pathogenicity of** *Alternaria altenata* **isolates on germination of cowpea seeds using the blotting paper fold technique**:

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The technology described by (ISTA ,1999) was used ,a 100 seeds of each studied plants were inoculated with three isolates of the fungus *Alternaria alternata*, the isolates were prepared from one week age of pure cultures of medium (PDA) supplemented with 5 ppm of pallas herbicide. Spores were added by adding 10 ml of sterile water to each dish with a diameter of 9 cm .The spores were harvested using a sterile glass rod and filtered through two layers of boring cloth .Then the concentration of the suspended spores was adjusted for each isolate of 1X 10^4 spores per ml using a Haemoycetmeter .100 seeds of the cowpea plant, spore suspension were divided into four groups (20 seeds for each replicate) and each group was immersed in the spore suspension of the previously mentioned fungal isolates, Coefficients were allocated for each isolate and laboratories for the fungal isolates treated with the herbicide Pallas ,after which the percentage of seed germination. according to a complete randomized design (CRD), the statistical analysis- Anova table of significant differences were calculated with L.S.D ($\boldsymbol{P} \geq$ o.o5).

5**. Effect of supplemented herbicide (pallas) with fungal isolates filtrates on seedling length of studied plants**

Isolates were prepared from pure cultures three isolates of the fungus *Alternaria alternata* then the method described by (Pitt, Hoking ,1997) ,which is summarized by growing them on a liquid media of Czapek Dox Broth supplemented with 5 ppm of pallas for 21 days during this period ,were prepared by gently shaking to grow rapidly and provide oxygen The fungal culture medium was filtered through a 1.Whatman No filter paper to get rid of the mycelium .The process was repeated three times ,after which the filter was passed through Milipore 0.45 pm microfilter Germinated seeds were prepared and superficially sterilized ,distributed among sterile filter paper saturated with sterile water and incubated at a temperature of 25 * 2 C^0 when the root length reached 1mm ,the germinated cowpea plant seeds were placed between two layers of sterile filter paper saturated with filters from each of the above fungi ,separately and by four replicates ,all isolates ,as a comparison treatment was prepared using a liquid medium that was not treated with any fungus .The cultures broth were incubated at a temperature of 25 * 2 C^0,and an exposure period was 12 hours .After five days ,the percentage of seedling growth inhibition was measured according to equation, Coefficients were allocated for each isolate and laboratories for the fungal isolates treated with the herbicide Pallas

The percentage to encouragment the growth of fungal isolates= The length of the seedling (comparison + treatment) \ The length of seedling in a comparison X100. according to a complete randomized design (CRD), the statistical analysis- Anova table of significant differences were calculated with L.S.D ($\boldsymbol{P} \geq$ o.o5).

6. Greenhouse tests:

The greenhouse experiment was conducted to verify the the pathogenicity test carried out in the laboratory, an inoculum of three *Alternaria alternate* isolates were used in inoculation technique.

Plants preparations:

The studied plants (cowpea) were planted in plastic pots with a diameter of 22cm ,with 4 plants in each pot ,irrigated with water and watched daily until the completion of germination and plant growth and the appearance of the first true leaf . Suspended isolates spores were prepared at a concentration of 1x10 ^{4} spores \ ml of inoculum of each of the fungi ,using one-week-old cultures on PDA culture medium ,and then leaves of plant crops were treated with spore suspension using small sprays for each isolate and separately .The plants were randomly distributed in the greenhouse according to a complete randomized design (CRD), the statistical analysis- Anova table of significant differences were calculated with L.S.D ($\boldsymbol{P} \geq$ o.o5). and after a month ,the following calculating were done:

1. The height of the plant.
2. Percentage of infection severity on the vegetative system according to pathological evidence .It consists of 5 degrees ,according to the following : 0 = healthy papers 1 = leaf spotted to a small degree only 2 = leaf spot by 25 % 3 = 50 % leaf spot 4 = leaf spot by 75 % 5 = 100 % leaf spot And then calculate the severity of the injury of each replicate according to the equation :

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Percentage of injury severity = (Number of plants by degree 0x0) +…+ (Number of plants by degree 5x5) \(The total number of plants examined X5) X10

Then the plants were carefully uprooted and washed with running water to remove the stacking soil, the plants were dried in the oven at 60C for 48 hours and the following was calculated to the roots and it was calculated:

3. Dry weight of seedling.

III. Results and discussion

8. Isolation and identification of fungi accompanying the leaves of infected plants.

The results of isolating and diagnosing the fungi associating with infected leaves of studied plants taken from (11) samples representing four areas of maize production in Babylon Governorate, namely Al-Hilla, Al-Hashimiya, Al-Kifl and Al-Musayyab, showed a variance in the isolated fungi. 17 species of fungi were isolated, the most frequent of which was the *Alternaria altenata* fungus. Therefore, three coded isolates showed table 1. were selected from different sites for subsequent tests:

Table 1. Isolates used in this study

No.	location	Fungus name	Code
1	Al-Hashimiya	*Alternaria altenata*	Aa 1
2	Al-Musayyab	*Alternaria altenata*	Aa 2
3	Al-Kifl	*Alternaria altenata*	Aa 3

9. **Pathogenicity test:**

All tested fungi significantly reduced the percentage of germination of radish seeds, Table 2. Germination ratio was ranking relativity first isolate effect on seed germination was Aa 1. with 51 % , followed by Aa 2. with 60 %, then isolate Aa 3, 69 %, compering 93 % in control treatment.

Table 2. Effect of *Alternaria altenata* isolates on seeds germination of radish seeds

No.	isolate	Germination ratio
1	***Aa 1**	**51**
2	**Aa 2**	**60**
3	**Aa 3**	**69**
4	**control**	**93**

- Each number referring to four replication.
- L.S.D.= 6.335. $P \geq o.o5$

10. Effect Pallas herbicide on the pathogenicity of *Alternaria altenata* isolates on germination of cowpea seeds using the blotting paper method:

The results of Table 3. show that all tested fungal isolates were a significant reduction in the percentage of germination of cowpea seeds compared with the control treatment (without fungi), and isolate Aa 1 was the most effective isolate in the percentage of germination, losing 55%, then isolate Aa 2 68 % then isolate Aa 3. With a germination rate of 78%, compared to the comparison equation, in which the germination rate of maize seeds was 91%

Table 3. Effect of *Alternaria altenata* isolates supplemented with 5 ppm pallas herbicide on seeds germination of cowpea seeds in paper blotter technique

No.	isolate	Germination ratio
1	**Aa 1**	**55***
2	**Aa 2**	**68**
3	**Aa 3**	**69**
4	**Aa 1+ pallas**	**25**
5	**Aa 2+ pallas**	**37**
6	**Aa 3+ pallas**	**40**
7	**control**	**91**

- **Each number referring to four replication.**
- **L.S.D.= 7.472. $P \geq o.o5$**

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Pallas herbicide has a significant impaction due to addition of 5 ppm of Pallas herbicide to the pathogen isolate inoculum to increasing pathogenicity of all of *Alternaria altenata* isolates, the germination ratio of cowpea seeds treated with Aa 1 isolate were reduced from 55to 25 % Aa 1supplamentd with Pallas herbicide, the same impact was recorded in Aa 2 isolate and Aa 3.

11. **Effect of filtrate of fungal isolates filtrates on seedling length of studied plants**

Table 4. indicates that all filters from cultures of fungal isolates had a negative effect on the seedling length of cowpea plants. The results showed that isolate Aa 1. is the most inhibiting the growth of cowpea seedlings, and significantly the highest inhibition compering with all other treatments, followed by isolate Aa 2. significantly superior to the rest of the treatments and then to isolate A3. The inhibition rate in the first fungus was 60 % for the second fungus 69 % , 77 % growth of seedling, respectively, compared to the control treatment).

Table 4. Effect of *Alternaria altenata* isolates filtrates supplemented with 5 ppm pallas herbicide on seedlings growth of cowpea plant

No.	isolate	seedlings growth ratio
1	Aa 1	60*
2	Aa 2	69
3	Aa 3	77
4	Aa 1+ pallas	45
5	Aa 2+ pallas	50
6	Aa 3+ pallas	57
7	control	100

- **Each number referring to four replication.**
- **L.S.D.= 5.421. $P \geq$ o.o5**

Pallas herbicide has a significant impaction due to addition of 5 ppm of Pallas herbicide to the pathogen isolate inoculum to increasing pathogenicity of all of *Alternaria altenata* isolates, the germination ratio of cowpea seeds treated with Aa 1 isolate were reduced from 60 to 45 % Aa 1supplamentd with Pallas herbicide, the same impact was recorded in Aa 2 isolate and Aa 3.

12. Greenhouse tests:

The results showed in table 5 that all tested fungal pathogens isolates Aa 1, Aa 2 and Aa 3 a significant impact in cowpea plant in all parameters were calculated.

Isolate A1 topped the rest of the isolates in the effect on reducing the germination of cowpea seeds in pots, as the percentage of germination decreased from 93 in the control treatment to 67 %, as well as in reducing the length of the leaves from 19 in the control treatment to 13.4 cm. The percentage of infection severity was recorded With leaf spot symptoms of 32.7 %, the dry weight of the one-month-old seedlings decreased from 822 mg in the control treatment to 688 mg.

The isolate A2 ranked second position of pathogenicity for all growth criteria and severity of infection, while isolate Aa 3 ranked third position.

Table 5. Effect of *Alternaria altenata* isolates inoculum supplemented with 5 ppm pallas herbicide on seedlings of cowpea plant showing the germination ratio, high plant, virulence ratio and dry weight

No.	isolate	Germination ratio	Plant high/ cm	Virulence ratio	Dry weight / mg
1	Aa 1	67*	13.4	32.7	688
2	Aa 2	70	15.3	29.1	692
3	Aa 3	80	17.2	24.4	698
4	Aa 1+ pallas	52	9.4	41.3	464
5	Aa 2+ pallas	58	10.5	33.6	470

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6	**Aa 3+ pallas**	**67**	**12.6**	**31.6**	**483**
7	**control**	**93**	**19**	**0.0**	**822**
	L.S.D	**7. 133**	**3. 504**	**4.720**	**100.101**

- **Each number in table is refereeing to average of four replication.**

Table 5 is clearly showmen that 5ppm of Pallas herbicide has a significant impact in cowpea all parameters calculated due to addition of 5 ppm of Pallas herbicide to the pathogen isolate inoculum , Aa 1 isolate treated with 5 ppm pallas herbicide has more virulence, germination ratio was reduced from 67 in Aa 1 without 5 ppm of pallas herbicide to52 %, the high of seedling were reduced from 13.4 to 9.4 cm, the virulence of leaf spotting were increased from 32.7 to 41.3 % and dry weight decreased due to addition of 5 ppm of Pallas herbicide to the pathogen isolate inoculum from 688 to 464 mg.

Same impaction was observe due to addition 5ppm Pallas herbicide inoculum of Aa 2 ,Aa3 isolates.

IV. Discussion :

Rustles showing in this study were clearly indicate that pallas herbicide has a significant impact to increase pathogencity of *Alternaria altenata* isolates.

This results are confirm with many studies that found that pathogence can using herbicides ingredients as nutrition agents to sporting pathogenicity factors like enzyme such as cellulose, pectinase and to producing toxins which they can destruction plant tissues, it is known that *Alternaria altenata* has necrotrophic pathway in parasitism activities.

Pallas herbicide is one of worldwide herbicide has sub- specification impaction in precise specialization weeds of plant species. it has been used a lot in agricultural fields in large areas in Iraq and the province of Babylon, especially in the winter season in the fields of wheat and barley, but the small concentrations and residuals of this pesticide remain in the soil of these fields and there may be a conflict with the summer crops that are grown In the same spaces as the cowpea plant

The *Alternaria altenata* isolates are common soil fungi spread in Iraqi soils, and they possess a sophisticated extracellular enzyme system that can analyze the spraying residues with this herbicide and its low concentrations can be turn it into a food substance that can grow on it and enhance its disease capabilities and attack many plants and Which is the cowpea plant.

Most of the herbicides leave many negative effects on economic and non-target plants and cause weakness in the growth of these plants and an imbalance in the immune system of these plants, Which makes it vulnerable to attacks of weak and medium pathogens and can turn into dangerous pathogens for these plants.

Several publications indicated the ability of many pathogens to growin vitro on medium prepared with low concentrations of herbicides from different groups with high efficiency (Duke et al., 2007; Sanyal & Shrestha, 2008).

Many studies revealed to important role of herbicide in increasing diseases severity in plants (Larson et al., 2006), it seem that is direct and in direct mode of action but the accurate mechanism still un clear yet (Lancaster et al. 2010).

The optimal use of herbicides and good management of herbicides are among the important factors that greatly affect the growth and production of economic plants, especially cowpea, and can provide them large scale of protection against fungal pathogens attack, on the contrary, the indiscriminate and wrong use of herbicides increases the attacks and spread of pathogens from fungi on economic plants.

v. Conclusion:

1. The *Alternaria altenata* isolates have a wide sapeartion in Babylon fields crophng with cowpea plants.
2. Low concentration of Pallas herbicide has large impact in influencing pathogenicity of The *Alternaria altenata* isolates cowpea plants.
3. Pallas herbicide may has impact in weakness of growth and an imbalance in the immune system of cowpea plants

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VI. Recommendation

1. Conducting more of research and studies about herbicides to figure out the impaction on pathogenicity of fungal pathogens on important crops.
2. Using accurate of concentration of herbicide to avoidance side effect of herbicides.

VII. Reference

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