Effect of UV-C and *P. Fluorescens* on Residual Rate of Herbicide Lintur 70% WG in Soil and Wheat Seeds

Adeeb K Al-Shafiee[1*], Jamal H. Kadhim[2], Salwan A.Z.J. Allwbawi[3]

Abstract

This study aimed to test the effect of two factors, biological (Bacillus cereus) and physical (UV-C) in degrading of herbicide Lintur residue on wheat plants. Pathogenicity in vitro experiments showed that *Pseudomonas fluorescens* is not pathogenic on wheat seeds. On the other hand, Lintur had negative effect on bacteria in solid and liquid media. As for the field experiments with regard to Lintur residual in the soil, the results showed that the highest concentration of the herbicide was 7.00 ppm in the treatment of uncultivated soil sprayed with Lintur, while the lowest concentration (below the detectable level) was in the wheat planted soil sprayed with UV-C treated Lintur in the presence of *Pseudomonas fluorescens*. As for the seed treatment, the highest active compound concentration was 1.90 ppm appeared in the Lintur treated seeds in the absence of other treatments, compared to the lowest residual concentration (0.00) ppm was in seeds treatment with UV-C treated Lintur.

Key Words: Alphacypermethrin, Pesticides, Physical Degradation, Toxic Substance, Wheat.
DOI Number: 10.14704/nq.2022.20.2.NQ22271 **NeuroQuantology 2022; 20(2):189-193**

Introduction

Chemical pesticide residues, their analysis and estimation of the minimum and upper limits of their residues is of importance in the global food trade to avoid the strict regulations that developed countries put in place to develop several methods of analysis for residues to achieve high levels of accuracy (Barceló, 2004). Studying chemical pesticide residues is an important issue in developing countries, including Iraq, because most farmers in these countries do not adhere to the recommended concentrations or the waiting period after treatment to ensure the product's eligibility for consumption. (Al-Ghazi et al., 2011). Studies indicate that despite the effectiveness of pesticides in controlling pests, the duration of these pesticides remaining in the soil may affect subsequent crops in a way that may lead to reduced crop growth or sometimes death completely (AL Mutlaq et al., 2003). Therefore, the vast majority of pesticides remained without studying the estimation of the residues, not to mention the modern pesticides that may be accompanied by information of this type, which may not be sufficient and at other times or uncertain unless confirmed by independent research institutions.

Ultraviolet rays, are electromagnetic waves with a wavelength shorter than visible light, as the wavelength of violet is the shortest among the colors of the spectrum with a length of 100 - (400 nm. The absorption is obtained at the wavelength (250-300) nm.

Corresponding author: Adeeb K Al-Shafiee
Address: [1*,2]Department of Plant Protection, Faculty of Agriculture, University of Kufa, Iraq; [3]Plant Protection Directorate, Ministry of Agriculture, Iraq.
[2]E-mail: jamal.alsharmani@uokufa.edu.iq
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 15 January 2022 **Accepted:** 04 February 2022

The photolysis of the UV rays is determined by some factors including, water molality, polarity, absorption coefficient, violet light absorption, water pH, and water solubility (Aaron and Oturan 2001). In regards to environment, UV radiation has been suggested as an effective means for removing residual herbicides from water (Chen et al., 2009). This study aimed to test the effect of biological factor (*Pseudomonas fluorescens*) and the physical factor (UV-C) in breaking down residue of the herbicide Lintur in wheat seeds and soil of the treated crop field.

Materials and Methods

The insecticide Flash 10% was obtained from the Abbasiya Agriculture Division, and seeds of a local wheat variety were used in the field experiment and to test bacterial pathogenicity. As for bacteria growth culture, Nutrient Agar and Nutrient Broth media were used, to prepare the bacterial suspension. Both media were prepared according to the instructions of the producing company (Difco, U.S.A). To confirm the viability of the seeds used in the study, the percentage of seed germination was tested in 1kg plastic pots using sterilized wheat seeds, with 10 seeds per pot and three replicates, irrigated, and the germination percentage was calculated after 14 days of sowing. Similarly, the pathogenicity of *Bacillus cereus* was tested on wheat seeds in plastic pots. Wheat seeds were superficially sterilized in 2% sodium hypochlorite for three minutes. The seeds were washed several times with sterile distilled water. The seeds were socked in the activated bacterial filtrate at a concentration of 10^6. Then, the seeds were planted in plastic pots containing 1 kg of sterile soil with three replications, while untreated seeds were planted to serve as control treatment (Hussain (2009). After seven days, the percentage of germination, rotting seeds and germinated seedlings was calculated.

Effect of Herbicide Lintur 70%WG on Pseudomonas Fluorescens

Bioassay in Solid Medium Nutrient Agar (N.A)

The medium was prepared using the ready-made Nutrient Broth in a 150 ml flask, sterilized by autoclaving, and left to cool to room temperature. Then, 0.1 ml of B. cereus was added, mixed well in N.B. (for activating the bacteria) and incubated at 30°C for 48 hours. Then, 0.1 ml of the prepared bacterial suspension was taken and added to Sterilized N.A. medium with the addition Lintur 70%WG at recommended dose. Before solidification, the medium containing the bacteria and pesticide and the media containing only the bacteria were poured in 9 cm Petri dishes with three replications. After incubated for 48 hours at 30 °C, the numbers of bacterial colonies were counted (Clark, 1965). *P. fluorescens*.

Bioassay in Nutrient Broth (N.B) Liquid Media

Sterile NB liquid culture medium was used, distributed in 100 ml beakers, then the beakers were inoculated with 0.1 ml of *P. fluorescens* with or without Lintur 70%WG, three replicates for each treatment, and then incubated at 30 ± 2 °C for 30 days with shaking. Every 2-3 days. Then 0.1 ml of each flask was taken, and placed in dishes with N.A. culture medium. The number of bacterial colonies was counted after the dishes were incubated for 48 hours at 30° C, with three replicates.

As for the bacterial filtrates (toxic and non-toxic) they were filtered through two layers of Whatman filter papers, and the degraded pesticides were measured by HPLC to determine the extent of the decomposition of the pesticide in the presence of *P. fluorescens* (2021, Mahajan)

Field Experiment

The experiment was carried out on 25.11.2020 in the Abbasiya district / Najaf governorate / Iraq on a wheat field with an area of 10750 square meters divided into 7 experimental units, each unit 2500 square meters. The treatments included spraying the insecticide Lintur 70%GW exposed or not exposed to infrared rays, treating the seeds before planting with *P. fluorescens* at a concentration of 10^6 in addition to the untreated control treatment.

Results and Discussion

Wheat Seeds Viability

The results showed that the seed germination percentage of the local variety wheat was 100% with high vitality of wheat seeds.

As for the effect of the bacteria (*P. fluorescens*) on the germination and rotting of wheat seeds, the results showed (Table1) that the presence of bacteria did not affect the germination rate as no seed rot or seedling death was recorded in any of the treatments. This may be due to the fact that these bacterial strains may secrete Gibbereline and Indole acetic acid (Hajjaj and Mohammed, 2001), which stimulate seed germination. In addition to various mechanisms shown by bacteria to mutualize with the host plant by producing antibiotics, and inducing systemic resistance (Bargabus et al., 2002). It has been reported that some bacterial species produce plant hormones, especially gibberellin, which has an important effect on seed germination and control of the germination process, in addition to the production of amylase enzyme, positively affecting seed germination (Ajayi and Fagade (2006).

Table 1. Effect of *P. fluorescens* bacteria on seed germination and seedling growth of wheat after seven days on N.A. at 30 ±2°C

Treatment	Seed germination%	Rotten seeds%	Infected seedlings%
Control	100	0.0	0.0
P. fluorescens	100	0.0	0.0

P. fluorescens Ability to Survive in Media Treated with Herbicide Lintur

Measuring Number of P. fluorescens Colonies

It was found (Table 2) that the highest number of bacterial cells (40.18) on the solid medium was recorded in the treatment of Lintur + *P. fluorescens* + UV compared to the lowest number (33.99) recorded in the control treatment of *P. fluorescens*. Also, in the case of liquid medium, the results showed that the highest number of bacterial cells 42.45 appeared in the full interaction treatment of Lintur + *P. fluorescens* + UV, while the lowest number of 38.57 was in the control treatment.

Table 1. Effect of herbicide 'flash' on *P. fluorescens* colony numbers on solid NA and liquid NB media

Treatments	**Bacterial growth CFU on culture medium**	
	NA	**NB**
Control	**33.99**	**38.57**
Lintur	**37.06**	**39.78**
Linture+UV	**40.18**	**42.45**

Although the pesticide is specialized on the bush, so the lack of effect of the pesticide on the growth of *P. fluorescens* may lead to the nature of the bacterial cell wall, which is semi-permeable and the molecules entering through it into the bacteria cell must be non-polar and soluble in fat (Baqer et al., 1989), so the pesticide molecules Most of the herbs are polar, soluble in water, and cannot penetrate the cell membrane (Shaaban and Al-Mallah, 1993). The results showed that the herbicide Lintur stimulated the growth of *P. fluorescens* bacteria in N.A. culture media. This may be due to the herbicide carrier substance contents of nitrogen and carbon, which the bacteria need as nutrients for bacterial growth and reproduction. Wasi et al., 2008 stated that *P. fluorescens* has the ability to thrive and degrade most water pollutants. These results are in agreement with the findings of Abdel-Jalil (2004) on the possibility of the growth of *P. fluorescens* bacteria in agricultural media treated with fungicides and the possibility of using these bacteria in integrated control programs. Also (Nawab, 2003) indicated that pseudomonas strains have the ability to degrade the residues of the commonly used organochlorine pesticides, DDT and DDE. The study found that the percentage of pesticide residues in untreated soil was three times higher than in soil treated with pseudomonas isolates.

Effect of P. fluorescens presence on UV treated Lintur 70% WG concentration

The results showed that the concentration of the active substance was significantly higher in the control treatment that did not contain bacteria. While, the lowest value of the concentration of the active substance of the herbicide Lintur was in the treatment of *P. fluorescens* bacteria on the N.A. medium which was significantly lower than that from the control (Table2).

Table 2. Effect of *P. fluorescens* presence and UV treatment on concentration of active compounds of Lintur 70% WG in NA medium

Treatments	Concentration of active compound ppm		Average
	Dicamba	Triasulfuron	
Control	33.20	24.90	29.05
P.flourecens	7.90	3.60	5.75
Average	20.55	14.25	
L.S.D.0.05	Treat. 3.88	Active comp. 2.54	Interaction 6.55

(Shahid et al., 2022) used 15 isolates of Pseudomonas bacteria isolated from the rhizosphere contaminated with agricultural chemicals. They were tested on 12 different chemicals (chemical pesticides) and 14 antibiotics, and Pseudomonas showed maximum tolerance for all the agrochemicals under study. The microbial enzymatic ability is effective in destroying pesticides, in addition to the fact that microorganisms cost less than the use of chemicals.

Where microbes work to break down pesticides and consume carbon and sulfur, microbes are used to reduce the toxins of chlorinated pesticides, polycyclic aromatic hydrocarbons, organophosphorous pesticides and others (kumari et al., 2022).

Effect of P. fluorescens and UV on the Concentration of the Herbicide Lintur in Post-harvest Wheat Seeds and Soil as Measured by HPLC

It was found from the results of Table (3) that the concentration of the active substance appeared in the highest concentration of the active substance Triasulfuron and less than that of Dicamba. In general, the highest concentration of the active substance appeared in the treatment of uncultivated soil sprayed with Lintur. The lowest concentration appeared in treatments of soil planted with wheat sprayed with Lintur treated with (UV-C) with or without the presence of bacteria. These results are in agreement with previous findings (Thalassinos & Antoniadis, 2021), that agricultural lands, especially those where wheat is grown, near intense human activities may be loaded with toxic elements, leading to increased risks to human health.

Table 3. HPLC results for the Effect of *P. fluorescens* and UV on the concentration of Lintur residues in post-harvest wheat soil

Treatments*	**Active subs. Conc. (ppm)**		**Average**
	Dicamba	Triasulfuron	
SS	6.50	7.50	**7.00**
LW	3.40	3.90	**6.65**
UV/LW	0.00	0.00	**0.00**
Pf/LW	0.80	1.20	**1.00**
Pf/UV-FW	0.00	0.00	**0.00**
Average	1.52	1.80	
L.S.D.0.05	**Treat. 0.173, Active subs. 0.092, Interaction 0.244**		

*SS, LW, UV/FW, Pf./FW, and Pf./IRFW are the treatments: Sprayed uncultivated soil, Lintur sprayed Wheat plants, Wheat plants sprayed with UV treated Lintur, *P. fluorescens* treated wheat plants sprayed with Lintur, and *P. fluorescens* treated wheat plants sprayed with UV/ Lintur, respectively.

As for the concentration of the pesticide in wheat seeds, the results (Table4) showed that the concentration of the active substance Triasulfuron was higher than that of Dicamba. Similar to the concentration of the pesticide in the soil, the highest concentration of the active substance appeared in the seeds of plants treated with Lintur only. While the lowest concentration was in Lintur treated with (UV-C). Note that the permissible limits are according to the European Food Safety Authority (EFSA) for Dicamba = 2ppm and for Triasulfuron = 0.05.

(AL-Anazy et al. 2021) explained that the use of ultraviolet rays led to the removal of 72-94% of the contaminated pesticides from fresh carrots, and ultraviolet rays could be used in the simultaneous decomposition of pesticides and microorganisms as a non-chemical and residue-free technique for disinfecting the surfaces of fresh products. On the one hand, a study (Li et al. 1969) showed that ultraviolet radiation led to the decomposition of 96% of the pesticides contaminating milk.

Table 4. HPLC results for the Effect of *P. fluorescens* and UV on the concentration of Lintur residues in post-harvest wheat seeds

Treatments*	**Active subs. Conc. (ppm)**		**Average**
	Dicamba	Triasulfuron	
LW	1.80	2.00	**1.90**
UV/LW	0.00	0.00	**0.00**
Pf/LW	0.91	1.13	**1.02**
Pf/UV-FW	0.00	0.00	**0.00**
Average	0.60	0.66	
L.S.D.0.05	**Treat. 0.319, Active subs. 0.260, Interaction 0.415**		

*LW, UV/FW, Pf./FW, and Pf./IRFW are the treatments: seeds of Lintur sprayed Wheat plants, seeds of Wheat plants sprayed with UV treated Lintur, seeds of *P. fluorescens* treated wheat plants sprayed with Lintur, and seeds of *P. fluorescens* treated wheat plants sprayed with UV/ Lintur, respectively.

References

Aaron, J.J., & Oturan, M.A. (2001). New photochemical and electrochemical methods for the degradation of pesticides in aqueous media. Environmental applications. *Turkish Journal of Chemistry*, *25*(4), 509-520.

Al Mutlaq , K.F. , C.M. Smith and D.A . Bal. 2003. Procarbazone – sodium effect on rotational crops and its dissipation in soils. Res. Bult., No. (120), Agric Res. Center, King Saud Univ. pp. 5- 29.

AL-Anazy, M.M., Alharbi, K.R., & Altowyan, A.S. 2021. Impact of Photolysis and TiO2 on Pesticides Degradation in Wastewater. Water 2021, 13, 655.

Al-Ghazi, Kadhimiya Wali Mansour, Lama Jassim Muhammad Al-Anbar, Hadiya Mawla Guam Al-Mayah, Jassim Hussein Abdullah Al-Maliki, Abdul-Jabbar Nasser Hamad, and Anwar Abdul-Karim Jumaa 2011. A study on the natural and unnatural use of pesticides in the rivers and marshes of Basra Governorate. Babylon University Journal. Pure and applied sciences. Volume 19 - Issue 1 - Page 142.

Baqer, Abdul Wahed and Luzan Amin Ali and Anis Malik Al-Rawi. (1989). bacteria. Ministry of Higher Education and Scientific Research. Baghdad University. House of Wisdom Press.

Barceló D. june 2004 Comprehensive Analytical Chemistry. Wilson and Wilsons. p.xxiii.

Bargabus, R.L.; N.K. Zidack.; J.W. Sherwood and Jacobsen. B.J. 2002. Characterization of systemic resistance in sugar beet elicited by a non- pathogenic, phyllosphere-colonizing *Bacillus* mycoides. Biological Control Agent. Physiol. Mol. Plant Pathol.,61:289-298.

Chen, F.; Wang, M.; Zheng, Y.; Luo, J.; Yang,X. and Wang , X. 2009. Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber *Fusarium* wilt by *Bacillus subtilis* B579. World Journal of Microbiology and Biotechnology 26:675-684.

Clark, F.E. 1965. Agar–pats method for total microbial Count. Madison. Wisconsin. USA. pp.1572.

Deng, Y., Liu, R., Wang, Z., Zhang, L., Yu, S., Zhou, Z., & Diao, J. 2021. The stereoselectivity of metconazole on wheat grain filling and harvested seeds germination: Implication for the application of triazole chiral pesticides. *Journal of Hazardous Materials*, *416*, 125911.

Hajjaj, Wafaa Muhammad and Muhammad Saber Muhammad. 2001. Use of industrial organic fertilizer loaded with hag-stimulating root bacteria to combat root rot disease of some vegetable crops grown inside greenhouses. Newsletter for Plant Protection in the Arab Countries and the Near East (FAO). Arab Society for Plant Protection. Beirut. Lebanon.

Hoesain, M., Prastowo, S., Pradana, A.P., Asyiah, I.N., Kustiawan, F., Ramadhan, F.S., & Nengtyas, Y. A. 2021. In vitro studies on Bacillus sp. and Pseudomonas sp. compatibility with botanical pesticide. In *IOP Conference Series: Earth and Environmental Science,* (Vol. 759, No. 1, p. 012069).

Kumari, S., Kumar, D., & Khurana, S.P. 2022. Microbial degradation of pesticides: microbial potential for degradation of pesticides. In *Development in Wastewater Treatment Research and Processes* (pp. 41-67). Elsevier.

Li, C.F., & Bradley Jr, R.L. 1969. Degradation of chlorinated hydrocarbon pesticides in milk and butteroil by ultraviolet energy. *Journal of dairy science*, *52*(1), 27-30.

Mahajan, R., Verma, S., & Chatterjee, S. 2021. Biodegradation of organophosphorus pesticide profenofos by the bacterium Bacillus sp. PF1 and elucidation of initial degradation pathway. *Environmental Technology*, 1-9.

Nawab, A., Aleem, A., & Malik, A. 2003. Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by Pseudomonas strains. *Bioresource technology*, *88*(1), 41-46.

Shaaban, Awwad and Nizar Mustafa Al-Mallah. 1993. Pesticides, Dar Al-Kutub for printing and publishing. University of Al Mosul .

Shahid, M., & Khan, M.S. 2022. Tolerance of pesticides and antibiotics among beneficial soil microbes recovered from contaminated rhizosphere of edible crops. *Current research in microbial sciences*, *3*, 100091.

Thalassinos, G., & Antoniadis, V. 2021. Monitoring Potentially Toxic Element Pollution in Three Wheat-Grown Areas with a Long History of Industrial Activity and Assessment of Their Effect on Human Health in Central Greece. *Toxics*, *9*(11), 293.

Wasi, S., Jeelani, G., & Ahmad, M. 2008. Biochemical characterization of a multiple heavy metal, pesticides and phenol resistant Pseudomonas fluorescens strain. *Chemosphere*, *71*(7), 1348-1355.