**Pseudomonas fluorescence Bio -compatibility with chemical fungicide Carboxin 75 and Raxil 2DS to control corn seedling blight causing by Fusarium graminearum, F. moniliforme and F. poliferatum**

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**Abstract.** This study was carried out for the purpose of detection and compatibility of *Pseudomonas fluoresce* and fungicides to control *Fusarium graminearum*, *Fusarium moniliforme* and *Fusarium poliferatum*. The pathogenicity in filter blotter test indicated that the seed decay symptoms were reduce from corn seeds to 52 for the fungus *Fusarium graminearum*, 55 for *Fusarium moniliforme* and *Fusarium poliferatum* 4.5 × 10⁶ was the lowest concentration showed inhibition of fungi growth of 77.5, 77.5, 85% sequentially. No toxic effects of chemical pesticides were recorded on the growth of P.f bacteria when mean concentration were used, the results showed a high degree of compatibility of *Pseudomonas fluoresces* bacteria with half effective concentration of chemical pesticides to suppressing pathogenic fungi, as well as an increase in plant growth parameters.

**Key words:** *Pseudomonas fluoresces* Bio- compatibility*Carboxin* Raxil

1. **Introduction**

Fusarium species cause severe damage to maize crop through the failure of seedling germination problems as well as fungal toxins that render the product unsuitable for consumption or trading *Munkvold [1]*. Pesticides are the most important elements in the management system of maize production. Pesticides can give a satisfactory and rapid results when used in maize fields, but in other hand its can left behind a lot of residues, which impact on soil fertility, soil microbial organisms and environmental effect *Komarek[2]*. Balance using chemical pesticides has become an urgent need to ensure continuous and environmentally sustainable cultivation of maize fields *Jasim [3]*. 

There was a need to reduce the doses of fungicides used in the fields of maize cultivation and it was necessary to replace this reduction by introducing an effective resistance agent *Abd-Aljalil [4]* mixing the fungicides in half the recommended dose with the biological control agent was one of the modern methods which been recommended by plant protectant and *Pseudomonas fluoresces* one of major bio-agent used for this porpus. Many studies found that these bacteria have great compatibility with fungicides such as carbendazim and mancoze. b and
Azoxystrobin Ahila[5] The study aimed to detect the potential of *Pseudomans fluorescens* (P.f) with Carboxin and Rexil.

2. Materials and Methods:

2.1. Fungal isolates:

The studied fungi were obtained from yellow corn seeds from the autumn yield of different areas of Babylon Governorate.

2.2. The pathogenicity test

The technique described by ISTA (6) was used, the isolated fungus inoculum was prepared from its pure and young colony, the spores were harvested and the concentration was adjusted. $1 \times 10^4$ spore / mL, 100 seeds of maize seeds were introduced. Seeds to four groups (25 seeds per replicate).

2.3. The source and the activation of bacteria

*Pseudomonas fluorescens* were obtained from the Department of Life Sciences Faculty of Science - University of Kufa, CHAO represents a commercial product (powder). The Pf bacteria was activated by using liquid KB medium prepared by melting 20 g peptone, 2.5 g of KH2PO4, 6 g MgSO4 and 15 ml glycerol, then supplemented with 1 L of distilled water, the medium was sterilized at 121 °C, 15 bar / 1 in$^2$ for 15 Min., 1 mL of commercial Pf, and placed the flasks were shaking for 10 minutes, then incubated at 28 ± 2 °C for 48 hours Leben [7]. 10 KB medium agar plates were inoculated with 1ml of P.f. inoculum for each and incubated at 28 ± 2 °C for 48 hours. The dishes were then tested using UV light to detect the characteristic Pf fluorescence characteristic in a poor Fe element medium, that-called fluoresces Keig (8). Single colony was transferred to a 100 mL glass flask containing 50 ml of KB liquid medium and shaken for 10 min. then incubator at 28 ± 2 °C for propagation and obtain pure isolation from the bacteria and use in antimicrobial tests.

2.4. The P.f bacteria antagonism test against isolated fungi

The antagonism of P.f was tested against *Fusarium graminearum* and *F. moniliforme* and *F. poliferatum* used the double-pollination technique on the PDA medium, a series of Pf strains was prepared. The dishes were inoculated with 0.5 mm of young colony of fungi isolates with 0.1 ml of challenging bacterial suspension, 4 peripheral spots and three replicates for each fungus and all dilution were used. Three dishes were left as control treatment to each fungus and incubated at 28 ± 2 °C for 7 days Abd-Aljalil [4]. The amount of inhibition was then calculated according to Abbot equation mentioned by Shabaan [9]:

\[
\text{Di. of mean of on.treat.} - \text{di. of mean of treat} = \frac{\text{Inhibition ratio} = \text{X 100}}{\text{Mean of con. treat.}}
\]

2.5. Bio-compatibility between pesticides and P.f.

Raxil and Carboxin fungicides were amendment PDA medium with 12 mg / L (half of the active concentration), these concentrations were shown to be effective in previous tests to study its effect on growth of the bacterial inoculation which was effective in inhibiting the growth of the studied fungi. 1 ml of bacterial inoculation on PDA agar plates. Those plates were prepared with PDA medium containing the studied concentration of the first pesticide and the same for the second pesticide. Three dishes were left without a pesticide representing a comparative treatment and left to solidify. The incubator
was incubated in an incubator at ± 28 °C 2 hours for 48 hours depending on the number of CFU to matching with con. treat. as a basis for calculating the bio-compatibility of bacteria with chemical pesticides.

2.6. Glass House Tests

The efficiency of P.f bacteria to biological compatibility with the chemical fungicides in suppressing fungal pathogens was carried out in pots experiment in the plant protection dep. - College of Agriculture - University of Baghdad glass hose:

Three control treatments for studied pathogenic fungi were done separately; three pathogens with seeds coated by Carboxin (Ca)+ separately; the same three treatment to Raxil(Ri) were done; also there were Pf + Ca (half recommended dose) for the three studied fungi; Pf + Ri (half recommended dose) + pathogenic fungi.

10 days later germination was calculated, the growth parameter were calculated after 30 days.

2.7. Investigation of the numerical density of P.f in the interactions with chemical pesticides in the glass house experiment.

This test was carried out by checking rihzospher samples after 10 days, after the end of the experiment, the CFU were calculated using UV light.

2.8. statistical analysis:

All experiments were statistically analyzed using the full randomization design CRD by GenStat.1997 program

3. Results and discussion

3.1. Pathogenicity test

The results shown in table 1 revealed to *Fusarium graminearum*, *F. moniliforme* and *F. poliferatum* were reduced the corn seeds germination ratio significantly.

| Isolates           | Germination ratio |
|--------------------|-------------------|
| *F. graminearum*   | 52                |
| *F. moniliforme*   | 51                |
| *F. poliferatum*   | 41                |
| L.S.D. p ≥0.05     | 7.86              |

* Each number represent the mean of three replicate

3.2. Antagonism test of P.f bacteria

Results showed the effect of the resistance of three concentrations of P.f. $10^6$, $10^7$, $10^8$ in the fungal growth of fungal pathogens is *Fusarium graminearum*, *F. moniliforme*, *F poliferatum*. The $10^6$ most recent highest percentage of inhibition form 40 was 77.5, 77.5, 85, Figure 1:
Figure 1. antagonism test of Pseudomonas fluorescens (p.f) against pathogenic fungi

3.3. Pf Bio-compatibility with chemical pesticides

The results of the effect of two fungicide carboxin 75 and rexil 2 DS on growth of bacteria (Pf) expressed by CFU / ml test showed no significant differences between the growth of bacteria in half-recommended dose of Carboxin and Rexil separately in $4.8 \times 10^8$ and $4.3 \times 10^8$ CFU / ml respectively, as compared with its growth on the fungicide free medium of $4.5 \times 10^8$, there were no significant differences, table 2.

Table 2. the bio-compatibility of Pf with fungicides

| treatment     | CFU    | Mean of colony |
|---------------|--------|----------------|
| P.f + Ca*     | $4.5 \times 10^7$ | 8.68           |
| P.f + Ri      | $4.3 \times 10^8$ | 7.63           |
| P.f. only     | $4.5 \times 10^8$ | 8.65           |
| **L.S.D. p ≥ 0.05** |        | 1.13           |

*Each number represent the mean of three replicate

3.4. Testing the efficiency of the biocontrol agent and chemical pesticides in controlling the pathogenic fungi of maize seeds and seedlings

The results of this test showed that all the fungal pathogens tested Fusarium graminearum, F. moniliforme, F. Poliferatum, Table 3, significantly reduced the percentage of germination of maize seeds, reaching 40, 65.7, 70 for the above fungi and respectively with the comparison. It also had a significant negative effect on the percentage of the severity of the root cause and F moniliforme was the most significant, reaching 83%. While F. poliferatum was the least effective and 40% was treated with a standard treatment of 0%. This effect was evident in the longer rate of maize seedlings, which decreased significantly for F. graminearum and F. moniliforme. The highest effect was 28, 28 cm. The fungus F. poliferatum had the least effect among the fungus. The average length of plants was 33 cm compared with control treatment 40 cm. The same is true with the dry weight of the vegetative total and the root table 3.
Table 3. Compatibility between biocontrol agent, pesticides ability to control pathogens on maize seed

| Treatment          | Germination % | Intensity of infection% | Plant shoot weight | Root weight |
|--------------------|---------------|-------------------------|--------------------|-------------|
| control            | 85            | 0                       | 40                 | 0.396       | 0.047       |
| F. graminearum F.g| 40            | 69                      | 28                 | 0.120       | 0.015       |
| F.moniliforme F.m | 65.7          | 83                      | 28                 | 0.083       | 0.015       |
| F. poliferatum F.p| 70            | 40                      | 34                 | 0.146       | 0.026       |
| F.g + P. f        | 68            | 39                      | 36                 | 0.152       | 0.032       |
| F.m + P. f        | 84            | 49                      | 35                 | 0.149       | 0.030       |
| F.p + P. f        | 85            | 19                      | 38                 | 0.190       | 0.043       |
| F.g + Ca          | 61.3          | 45                      | 33                 | 0.136       | 0.024       |
| F.m + Ca          | 80.3          | 58                      | 32                 | 0.125       | 0.023       |
| F.p + Ca          | 77            | 22                      | 36                 | 0.179       | 0.033       |
| F.g + Ri          | 60            | 48                      | 33                 | 0.135       | 0.020       |
| F.m + Ri          | 80            | 59                      | 31                 | 0.124       | 0.021       |
| F.p + Ri          | 75            | 25                      | 38                 | 0.179       | 0.033       |
| F.g + P. f + Ca   | 71            | 36                      | 37                 | 0.165       | 0.036       |
| F.m + P. f + Ca   | 86            | 48                      | 36                 | 0.160       | 0.033       |
| F.p + P. f + Ca   | 86            | 18                      | 39                 | 0.200       | 0.045       |
| L.S.D. p ≥ 0.05    | 3.34          | 5.73                    | 1.47               | 0.015       | 0.005       |

* Each number represent the mean of three replicate

3.5. Investigation of the P. f inoculum density in the chemical pesticides treatments

The results shown in Table IV indicate no significant differences (P = 0.05) in the density of P. f when added to the chemical pesticides, which included P.f+ carboxin fungicide and P. f + Raxil fungicide when compared with the treatment of bacteria only. The results were based on the number of CFU in the dishes after 10, 30 days the same indicators were after 10 days in the treatment of bacteria only 2.7 × 10^8 CFU In the treatment of bacteria + Carboxin pesticide 2.6 × 10^8 CFU and in the treatment of bacteria + Raxil fungicide 2.5 × 10^8. After 30 days, it was 3.8 × 10^8, 3.7 × 10^8, 3.6 × 10^8 CFU respectively. This means that there is no negative effect of both fungicide on the growth of P. f, and therefore the possibility of using bacteria to integrate with these pesticides, thus reducing the amount of pesticides used in pest control

Table 4. effect of fungicides on the growth of P.f. represent ate by CFU

| Treat.       | CFU after 10 days | Mean of logarithmic of CFU | CFU after 30 days | Mean of logarithmic of CFU |
|--------------|--------------------|----------------------------|-------------------|----------------------------|
| P.f only     | 2.7×10^8           | 8.43                       | 3.8×10^8          | 8.57                       |
| P. f + Ca    | 2.6×10^8           | 8.41                       | 2.7×10^8          | 8.56                       |
| P.f+ Ri      | 2.5×10^8           | 8.40                       | 2.7×10^8          | 8.56                       |

3.6. graminearum produces many secretions and toxins that affect the vitality of the seeds.

Deoxynivalenol (DON) is the most important of these toxins, which plays an important role in causing Fusarium Head Blight (FHB) disease on the corn seeds in the field, reducing germination
and vitality of corn seeds. The mechanism effect of poison is to inhibit the production of protein in the cell, thus preventing the growth and division of the infected cells Rotter [10] as well as the secretion of many enzymes that brake down the plant tissues such as Lipase.

The tolerant of P. f to carboxin and relxil effect may be due to that these fungicides are specialized in fungi organism, The vitial pathway through which the fungicide can fertilize the fungal cell is not present in the bacterial cell at these concentration.

P.f. bacteria has the ability to break down carboxin and convert it into a source of nitrogen and carbon feed Pal [11]. These results are consistent with Abd-Aljalil [4] in the possibility of Pf growth in medium adamant with Redomil Hasaan [12].

Chemical pesticides may not affect the growth of P. fluorescens to the nature of the bacterial cell wall, which be covered with capsule and the molecules must penetrate this capsulesenter to bacterial cell Baqer [13]. Since the active ingredient of the fungicide is polycarbonate Shabaan[9], so the molecules of the pesticide cannot reach the cell membrane of bacterial cell.

The wide variation in germination and infectivity reflects the difference in the pathogenicity of these species of fungi, the nature of fungal secretions, as well as the susceptibility of fungi to the secretions of maize roots Glenn [14].

The superiority of F. moniliforme, the high rate of infection to maize seedling compared with relatively little effect of germination may be due to the nature systemic infection, the symptomless of this fungus which had little effect on germination stage and reappear strongly in the growth stage. Seedlings because of the ability of fungi to move from the seed tissue to the base of the stem, causing rotting to the base of the corn stalk, root rot seedling maize Munkvold [1]. The ability secretion of many secondary metabolic compounds such as Fayomazin, MoniliFormin, Fuzaric acid and other metabolic compounds, which play an important role in the pathogenic effect of F. moniliforme on the maize plant and many griminess plants Abbas [15].

From these results we conclude that biological treatment has significantly increased the overall performance of the chemical pesticides, pesticides have no negative affected on the performance of the bio-control bacteria. These results are consistent with Hassan[12] efficacy of carboxin in inhibiting the activity of R. solani, U. atrum, Fusarium sp in several oil crops. As well as with what Jones[16] found in the efficiency of the fungicide against Fusarium graminearum in wheat seeds. They also agree with what Shams-Allah [17] found in the efficacy of the Raxil herbicide in controlling the pathogenic fungi to Iraqi wheat.

Johnson [18] was found the efficacy of P. fluorescens against bipolaris oryza and B. sorkiniana on wheat and rice, and of the efficacy of P. fluorescens in inhibiting Fusarium graminearum on wheat plants. As well as the ability of P. f to inhibit the diseases of corn-root rot from controlling the fungal infection of F. graminearum, F. moniliforme on the root of the corn seeds.

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