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ISOLATING AND DIAGNOSING THE FUNGI RESPONSIBLE FOR PALM LEAF SPOT DISEASE IN CERTAIN ORCHARDS IN AL NAJAF GOVERNORATE, AND CHEMICALLY CONTROLLING THEM

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ABSTRACT

This study has been conducted in the Laboratory Association in the Directorate of Agriculture of Najaf Al-Ashraf. The study aims to isolate the fungi that present with the symptoms of palm leaf spot disease in certain orchards in Al- Najaf Governorate. The study indicates that various species of fungi have emerged through the process of isolation, including Fusarium oxysporum; Aspergillus sp; Alternaria spp; Alternaria alternate; Thielaviposis paradora; Cladosporium herbarum; Fusarium solani; Mucor sp; and Aspergillus spp. Furthermore, during the researchers' testing, it is discovered that isolated fungi such as Lind Gar Cladosporium herbarum, keissler Alternaria alternate, F.solani (Mort.)sacc, and Fusarium oxyporum schelecht possess the pathogenic capability to induce palm leaf spot disease. The results of an experiment have assessed the efficacy of two different chemical pesticides. It has been observed that the chemical pesticide Othello top 32.5% SC TM effectively reduces the rate of fungal infection. Specifically, after spraying in the orchards of Al-Mishkhab district, the infection rate decreased to 2.33%. In contrast, the infection rate remained higher at 20.67% after spraying in the orchards of Al-hira district. Similarly, the results of spraying Al Najaf district orchards with the chemical pesticide (Brak 24 EC) for infection severity show that the lower infection rate after spraying is %1.9. However, in the orchards of Al-Haydaria district, the infection rate increased to 18.5% after spraying. There is no significant difference in infection severity between the two types of pesticide used in the orchards of Al Najaf and Al-Mishkhab districts. Furthermore, the chemical pesticide (Othello top 32.5% SC ™) is more efficient in pesticide spraying. It achieves an efficiency rate of 85.1% in Al-Kufa orchards and a lesser rate of 61.66% in Al-Hira orchards. In addition, the chemical pesticide (Brak 24 EC) demonstrates a higher level of effectiveness in orchards located in the Al Najaf district, with an efficiency rate of 70%. Conversely, orchards in the Al Abbasia district show a lower efficiency rate of 57.27%. Besides, the chemical pesticide, (Othello top 32.5% SC ™), is superior in terms of spraying efficiency. It effectively reduces the percentage of pests in four different orchards, with an average spraying efficiency of 78.67%.

INTRODUCTION

Phoenix Dactylifera L is a member of the Arecaceae family of palms. Due to their high economic yield, palm trees are generally regarded as the most significant trees planted in the Arab world, particularly around the Arabian Gul Unfortunately, palm trees are highly susceptible to a multitude of insect and disease pests (Barbendi et al., 2000). Palm leaf spot infections are prevalent in many

regions where palm trees are cultivated worldwide. These diseases are caused by many forms of fungi, (Mohammed Abd al-Qader, 1997). In neglected orchards, the symptoms of the palm leaf spot disease manifest more extensively on the old leaves (fiber) than the new ones. Moreover, these symptoms vary based on the fungi that cause them or the environmental factors that contribute to them, there has been an emergence of additional fungi responsible for causing palm leaf spot diseases. These include the fungus Chaetosphaeriopsis sp (Mustafa et al., 1971), the fungus Mycosphaerella tassiana (Carpenter and Elmer, 1979), as well as the fungi Alternaria alternate, Phoma sp, Diplodia phoenicm, Stemphylium botryosuim, and Ulodadium atrum, (Abu Hayia et al., 1983; Kassimet et al., 1983). The palm leaf spot disease has recently been spreading throughout numerous orchards in Al Najaf Governorate, resulting in a rise in palm cultivation problems. As a result, the aim of this study is to isolating the fungi that are associated with the symptoms of palm leaf spot disease in specific orchards in Al Najaf Governorate. Besides, the study aims to assess the efficacy of two chemical pesticides that are currently use in field combat.

METHODOLOGY

The Survey Studies for Palm Leaf Spot Diseases

The researchers conduct the initial field survey during the 2021-2022 farming season, followed by a second survey during the 2022-2023 farming season. The surveys are conducted in four areas within Al Najaf Governorate: Haydaria, Munathera, Kufa, and Mishkhab. The surveys focuse on three different types of palm trees: Barhy, Zahdy, and Helawy, which are present during both survey periods. Moreover, this experience primarily emphasizes the broader range of symptoms, namely spots, black scorch, and dry palm fronds. The samples are gathered in Polyethylene bags, thereafter labeled, and stored in the Frederator at a temperature of 4 degrees Celsius.

Isolating and Diagnosing the Fungi Responsible for Palm Leaf Spot Diseases

The diseased plant fragments are cut into small 1 cm pieces and washed to remove any dust. Subsequently, the pieces are treated with a 10% concentration of sodium hypochlorite liquid for a duration of six seconds. Following this, the fragments are rinsed with distilled sanitizing water to eliminate any traces of sterilization.

Following the preceding procedures, the sanitizing pieces have been dried using sterile filter paper. Subsequently, all four fragments are transferred to Petri dishes with a diameter of 9 cm. These dishes contain Agar, a medium composed of Potato and Dextrose (PDA), which has been sterilized using an autoclave. Additionally, the antibiotic chloramphenicol is added to the medium at a concentration of 125 mg/L. Afterwards, the plates are transferred to an incubator set at a temperature of 25±2°C for a duration of 3 to 5 days. Following this, the fungi are isolated and purified on a PDA medium. Daily, the researchers observe, cleanse, and examine the growth of the fungus using a ZEISS compound microscope. Eventually, the fungi are identified based on the works of Pitt-Domsch et al. (1980), Ellis (1971, 1976), and Hocking (1997).

Pathogenicity Testing of the Isolated Fungi

The researchers employ the methodology described by Elmeleigi et al. (1986) to assess the pathogenicity of the isolated fungus. They select healthy leaves (wicker) that are of the same length as the third stage leaves of the Al-Zahdi date variety. The leaves undergo a process of water washing, followed by sterilization using 70% ethyl alcohol, and then another round of washing with sterilized distilled water. The leaves have been categorized into two groups: one group has been punctured with a sterile needle, while the other group remains unpunctured.

Two leaves are inserted in each Petri dish containing 10 ml of sterile distilled water, with three replications for each fungus. At a concentration of 10-6 spores/ml, the leaves are treated with a

spore suspension of each fungus from laboratory fungi using a hand spray (Atomizer). Next, the dishes are sealed and placed into the incubator at a temperature of 2±25°C. The researchers observe the onset of symptoms every two days for a duration of two weeks from the day of spraying. This includes a comparable treatment where punctured and unpunctured leaves are sprayed with distilled sterile water.

Quantifying the infection rate and severity of the Palm Leaf Spot Disease in orchards of Al Najaf Governorate pre- and post- pesticides spraying

The following equations have been used to calculate the infection percentage and severity of numerous palm tree species in Al Najaf Governorate, as estimates by the researchers:

$$\%100 \text{ infection} = \frac{\text{The number of infected palms}}{\text{Total number of palms}} \times 100$$

$$\text{The severity of infection} = \frac{\text{Total number of infected plants * its degree}}{\text{Total number of infected plants * higher degree of infection}}.$$

The severity of infection has been quantified using a six-degree scale, as indicated below:

Degree	Description
0	No spots on the leaf
1	+ 10 spots of the leaf
2	1+20 spots of the leaf
3	1+30 spots of the leaf
4	1+40 spots of the leaf
5	More than 40 spots of the leaf

The severity of infection is determined using the equation developed by Mickenny in 1923.

Ultimately, the data is statistically analyzed by L. S. D., and the results are compared using a polynomial test at the 0.05 level of probability.

The Percentage of Pesticide Spraying Effectiveness

The effectiveness of pesticide spraying in reducing the percentage and severity of palm leaf spot infection is assessed throughout the first and second seasons in orchards located in the areas of Al Najaf Governorate.

In the first season, the trees are treated with fungicides containing Othello top 32.5% SC TM, at a rate of 75 ml per 100 liters. In the second season, the trees are treated with fungicides containing Brik 24 EC, at the same rate of 75 ml per 100 liters.

The researchers utilize a portable method to carry out the spraying procedure on a car liter with a capacity of 500. However, the process of spraying the trees should continue until complete coverage is achieved. After a period of 60 days, the persentage and severity of infection, as well as the effectiveness of Pesticides in controlling it, are calculated. This is done for two seasons using the following equation:

$$\text{The effectiveness of infection} = \frac{\text{The rate and the severity of infection treatment}}{\text{The rate and the severity of infection treatment}} \times 100$$

RESULTS AND DISCUSSION

Classification The Findings of a Field Survey on the Infection of Palm Leaf Spot Disease Infection

Figure (1) depicts the percentage of infection and its severity in the orchards surveyed. The orchard in Al Hera district has the highest infection percentage at 60%, while the orchard in Al Mishkhab district has the lowest infection percentage at 15%.

Regarding infection severity, figure (1) indicates that the orchard in Al Hera district has the highest severity percentage at 54%, while the orchard in Al Mishkhab district has the lowest percentage at 14%.

Based on the research conducted by (Ghali, 2001; Al-Asadi, 2004there is a strong correlation between the occurrence and severity of palm leaf spot disease and the presence of accompanying fungi, as well as the sensitivity of different types of palm trees. Research indicates that certain varieties of palm trees, including Halawi, Zahidi, Barhi, and Sayir, exhibit greater sensitivity to fungal infections compared to other types, such as Barban and Barhi.

On the other hand, there is an inverse correlation between the severity of infection and the wax content on the leaves. As the wax content decreases, the infection of the Zahidi palm with the palm leaf spot disease becomes more severe, (Manah, 2004). Contrarily, there is a direct correlation between the neglect of palm tree orchards and the infection percentage of the palm leaf spot disease. It has been discovered that the infection percentage increases significantly in the orchards that are ignored.

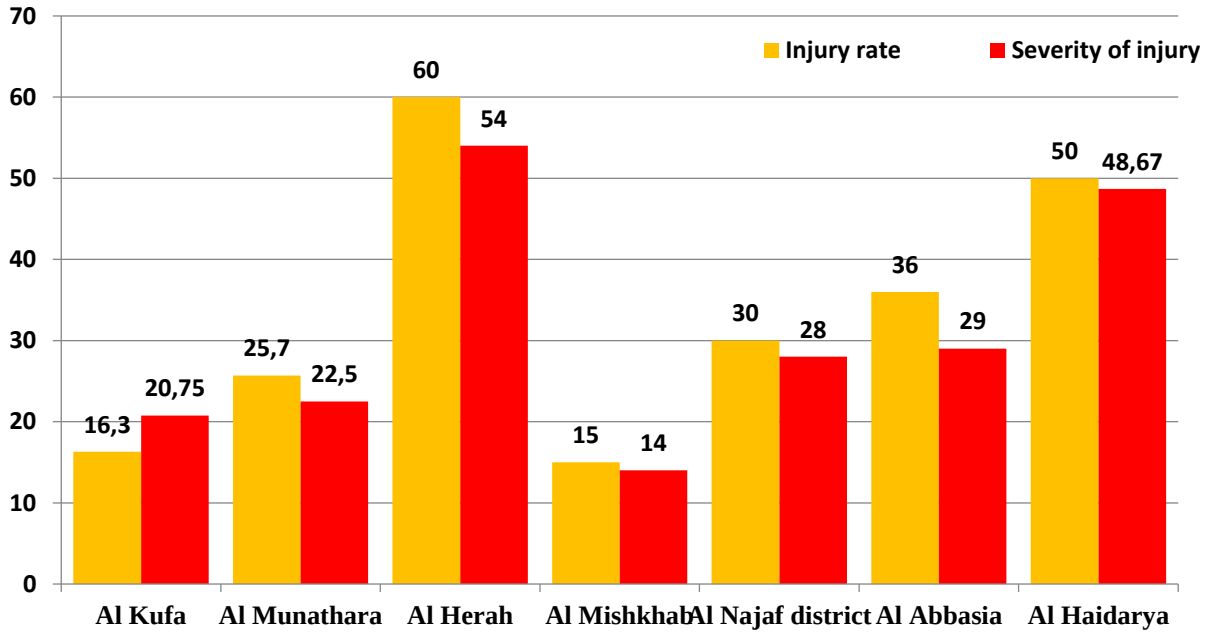

Figure (1) displays the findings from a field survey assessing the severity and frequency of infection with palm leaf spot disease in certain orchards located in Al Najaf Governorate

Isolating the Fungi that are Associated with the Symptoms of the Palm Leaf Spot Disease

The researchers of this study observe that the samples of palm leaves affected by the leaf spot disease display distinct brown spots of various sizes and types on the leaves, fronds, and thorns. As the infection progresses, the spots become darker and sometimes exhibit a yellow aura or are surrounded by dark rims. Furthermore, the findings from isolating the palm leaves affected with spot disease have revealed the presence of several fungi that are associated with the illness symptoms, as depicted in figure (1).

These findings corroborate the viewpoint of Abid Al Khader (1997) that a one spot is frequently associated by many fungi. In addition, several researchers have identified the fungus Phoma sp., Alternaria alternata, and Cladosporium herbarum as potential causes of palm leaf spot disease, (Carpenter & Elmer, 1978; Kassim et al., 1983; Abul-Hayja & Sheir et al., 1981).

Table (1) shows the isolated fungi with the symptoms of the palm leaf spot disease in Al Najaf Governorate

N	The fungi
1	*Alternaria alternate* (Fr.) Keissler
2	*Alternaria spp*
3	*Aspergillus sp*
4	*Aspergillus spp*
5	*Fusarium oxysporum* schlecht
6	*Fusarium solani* (mort.)sacc.
7	*Thielaviopsis sp*
8	*Cladosporium herbarum* (Pers.) Link 8 Gary
9	*Mucor sp*

The pathogenicity of the palm leaf spot disease

The pathogenicity test results (table 2) demonstrate that the fungi are capable of infecting both the punctured and unpunctured leaves. These findings are consistent with previous research that identified a combination of fungi as the cause of palm leaf spot disease, (Abd Al-Qadir & Mohammed, 1997; Fayyad & Manah, 2008). Furthermore, the test findings demonstrate variations in symptoms and their severity as a result of the different types of fungi that have been studied. Particularly during the second week of the infection, as seen in the aforementioned table.

Table (2) displays the pathogenicity exhibited by the infected fungi on the artificially pollinated palm leaves.

Fungi	The symptoms after a week	The symptoms after two weeks
Fusarium solani	Rounded dark brown spots which are densely spread on the leaves	Formation of a dark brown blotch as a result of a combination of the spots with each other the edge of the leaves
Fusarium oxysporum	Dark brown spots in the upper and lower side of the leaves	the edges of the upper leaves margins becoming stiff
Alternaria alternate	Separate dark brown spots on the middle and upper side of the leaves and coloration of the middle vein of the leaves with a brown color	Spreading of spots on the side of the leaves with coloration of middle vein of the leaves with a brown color
Cladosporium herbarum	coloration of the middle vein of the leaves with a brown color	coloration of middle vein of the leaves with a dark brown color with spots appearing on the lower side of the leaves

Quantifying the infection percentage and severity before and after the spraying insecticides in select orchards inside Al Najaf Governorate.

The test findings depicted in Figure (2) indicate that the Orchards of Al Mishkhab district exhibited the lowest infection severity percentage (2.33%) after being treated with Insecticide (othellotop), whereas the highest infection severity percentage (20.67%) is observed in Al Herah district after the spraying treatment. This suggests that there is a notable disparity amongst the districts, with the exception of Al-Kufa and Al Munathara, which have respective values of 4 and 2.47.

Conversely, the percentage of the lowest infection intensity in the orchards of Al Najaf district is (8.33%), while the percentage of the highest infection intensity in Al Haidarya district is (19%). This discrepancy is significant due to the fact that the insecticide (othellotop) is a combination of two active substances. The first substance, Azoxtytrobin 20%, has the highest active range among the currently known antifungals. The second substance, difenoconazole 12.5, is rapidly absorbed by the surface of the infected plant leaves and then spreads to the remaining plant parts via the

phloem vessels. Furthermore, it has a long-lasting impact and a high transitional movement, in addition to controlling a significant number of fungi. Nevertheless, the inclusion of these two substances in an insecticide result in a fast-acting, exceptional, and reliable insecticide that effectively reduces the infection rate, (Othello Top, 2015).

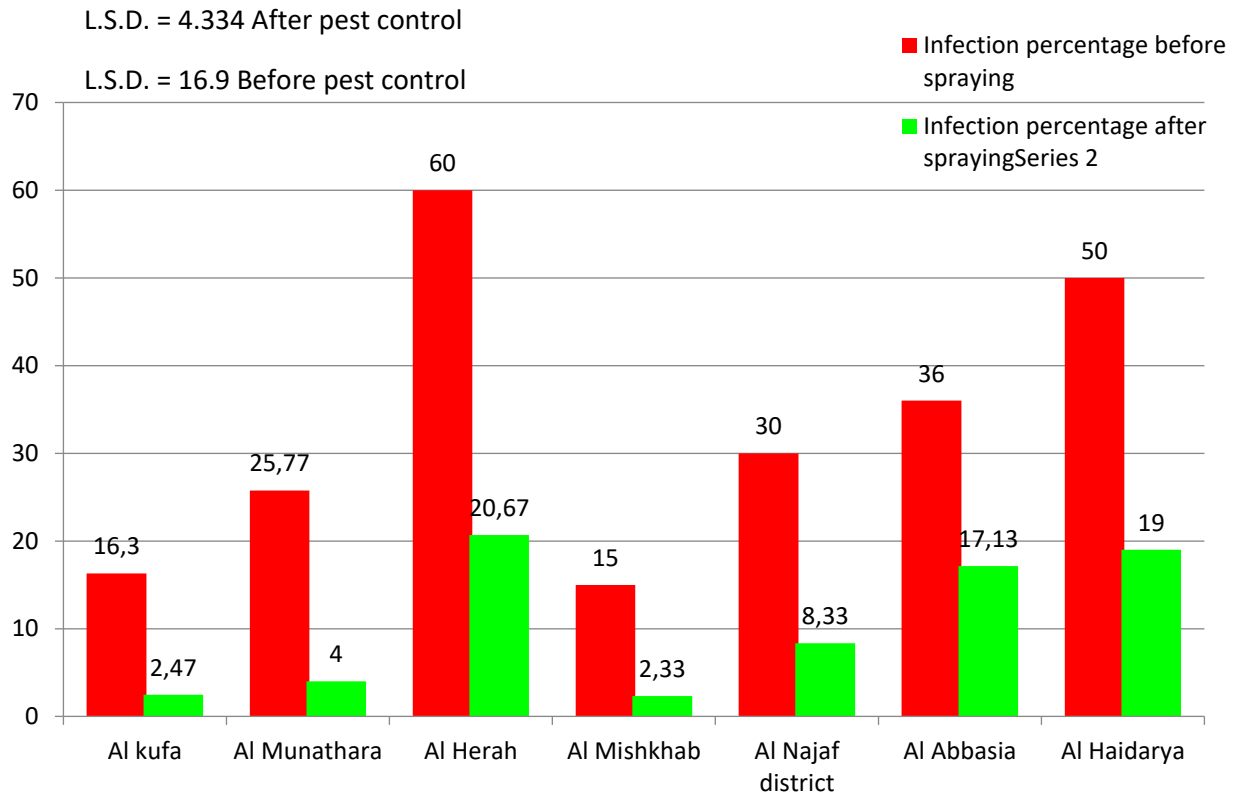

Figure (2) displays the infection percentage in certain orchards of Al Najaf governorate both before and after spraying the pesticides.

Quantifying the infection intensity percentage in select orchards within the Al Najaf governorate.

The findings in figure (3) demonstrate that the infection severity in Al Najaf orchards is the lowest at 1.9% after the spraying of Insecticide (brak), while the highest infection severity in Al Haidarya orchards is 18.5%. This disparity is substantial; however, it is insignificant in comparison to Al Abbasia Division. Conversely, the infection severity in Al Mishkhab district is the lowest at 3% after the spraying of Insecticide (othellotop), while in Al-Hera District it is the highest at 22.8%. The severity of the insecticides in Al Najaf and Al Mishkhab districts is not significantly different, which is attributable to the systemic pesticides that are used, which have the capacity to penetrate plant tissues either completely or locally. The xylem and bark vessels allow it to enter the plant tissues, or it can travel through the leaves without affecting the remaining plant tissues, (Abdel-Zaher, 2022).

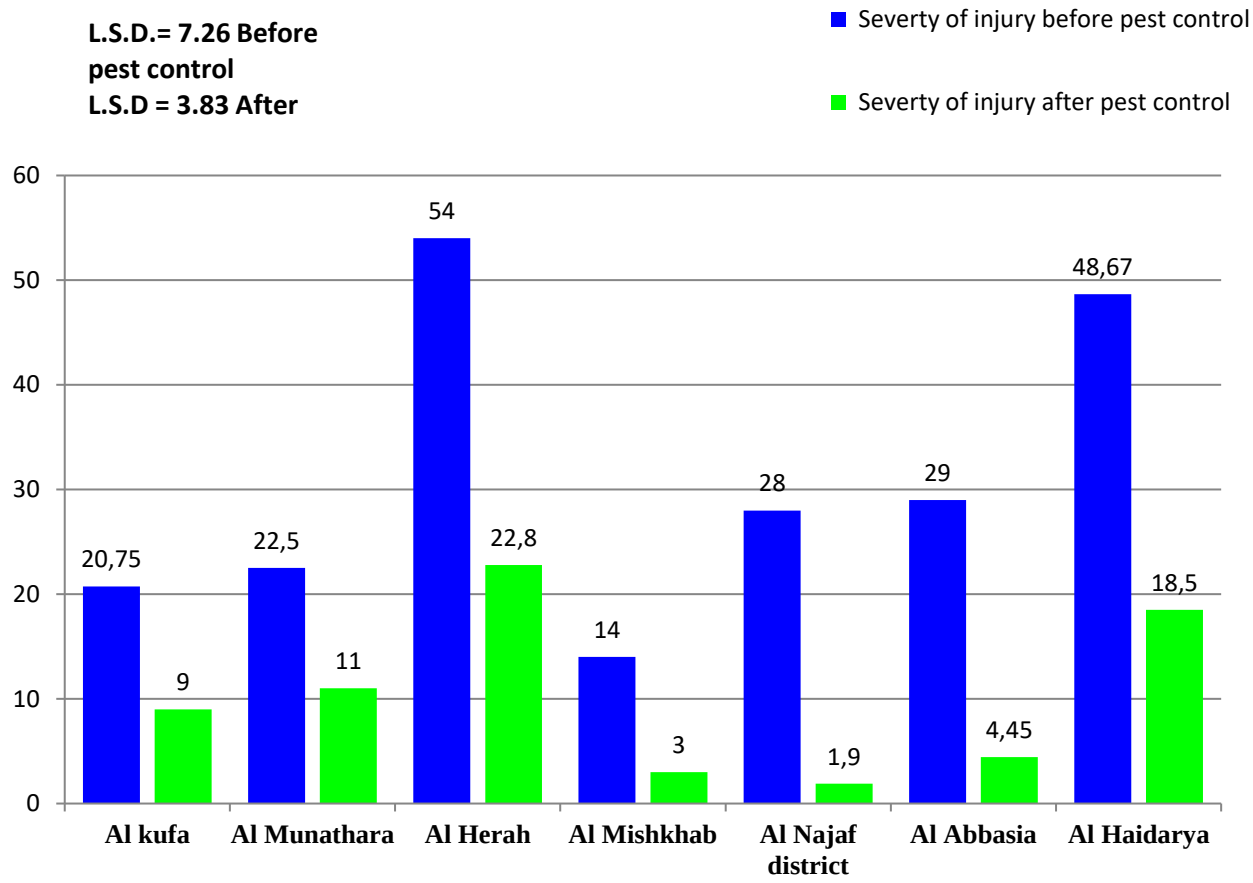

Figure (3) illustrates the calculation of the infection intensity percentage in specific regions of the Al Najaf governorate

Estimating the percentage of the insecticide spraying efficiency

The results indicate that the efficiency of the (Othello top 32.5% SC™) insecticide, when compared to the (Brik 24 EC) insecticide, is higher. Specifically, the spraying of the (Othello top 32.5% SC™) insecticide in four areas of orchards in Al Najaf Governorate resulted in a decrease of infection by 78.67%. The highest efficiency rate of insecticide spraying in Al Kufa orchards is 85.1%. On the other hand, the efficiency of the (Brik 24 EC) insecticide in three areas of the orchards in Al Najaf is 62.4%. The highest efficiency rate of insecticide spraying in Al Najaf district orchards is 70%.

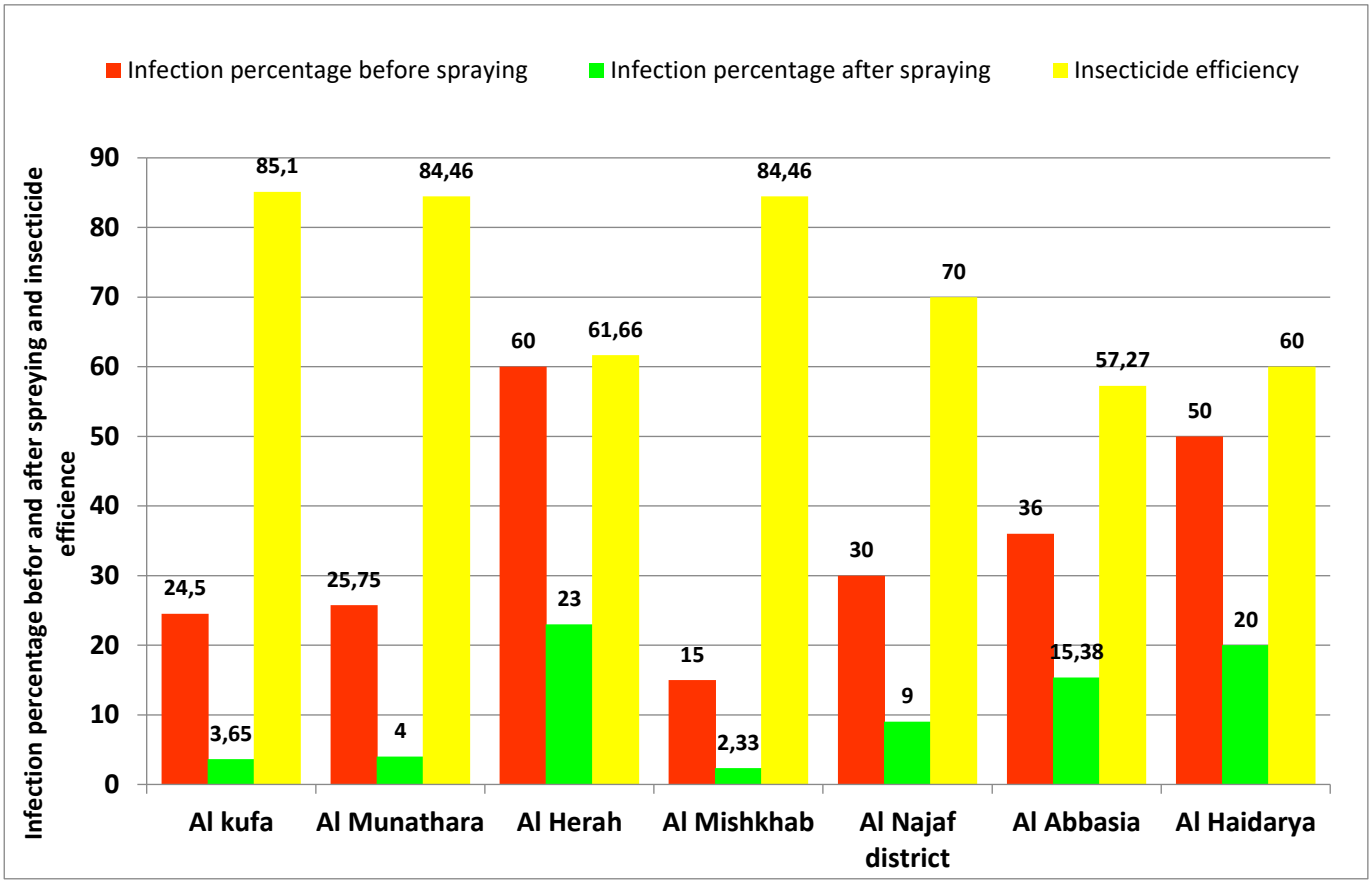

Figure (4) illustrates the estimation of the insecticide percentage efficiency at the infection percentage

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