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Efficacy of Some Fungal Seed Dressers in Controlling *Fusarium oxysporum* f. sp. *Cicer* on Chickpea under Artificial Infection Conditions

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Abstract: Seeds of chickpea cv. Ghab3 were treated with three fungicides; Oxycure, Topsin and Vitaflo, and infected by *Fusarium Oxysporum* f. sp. *Ciceri* at culturing under a glasshouse condition. The results showed that Oxycure (Oxychloride) caused a significant increase in seed germination compared with other treatments, but it could not provide this protection at the post-germination phase, resulting in a significant increase in seedling mortality to 42.1% at 15 days after germination, compared with the other fungicides. In addition, Topspin (Thiophanate-methyl) was an effective protectant at germination stage, and in the seedling one too, since the percent of seedling mortality did not exceed 11.11%, whereas, Vitaflo had the least effect in protecting seeds from fungal infection, but with a significant difference with the positive control, as the percentage of non-germinated seeds was 41.66% and 62.5%, respectively.

Key words: Chickpea, *Fusarium oxysporum* f. sp. *Cicer*, fungicides, sterilizers, Syria.

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third legume crop worldwide, after bean *Phaseolus vulgaris* L. and peas *Pisum sativum* L. [1], India is the first country by 75% of cultivated area [2] and 90% of production [3]. In Syria, the cultivated area of chickpeas is 10,200 ha, and the annual production is estimated as 88,800 tons [2].

The most common disease that affects chickpea yield, other than Ascochitablights *Ascochytarabiei*, is *Fusarium* disease caused by fungus *Fusarium oxysporum* f. sp. *Ciceri* which has great importance in India, Iran, Spain, Tunisia, Mexico, Syria and Pakistan [4], and it causes a loose up to 60% of production [5], and the field can be destroyed when infected at Phases of vegetative growth or podding [3, 6, 7], as India in 1973 [8].

The infection occurs at the stages of seedling, flower in gorpodding [9], but the highest incidence is at the later ones, if associated with a sudden rise in temperature and water stress [10]. The fungal mycelium penetrates the host roots to the xylem tissues, and spreads quickly through the vascular system, causing block to water and nutrients flow, that results in wilting and leaves death, and is often followed by death of whole plant [11].

This disease is primarily managed using resistant varieties, but the variation and high ability to form mutations in pathogens limit the sustainability and effectiveness of any naturally selected resistance [12]. In the current context, fungus *Trichoderma harzianum* is an effective biological agent successfully used to suppress Fusarium wilt [13, 14]. Similarly, soil treatment with plant extracts greatly reduces Fusarium wilt in the field [15]. However, the biological control of plant diseases is often regulated by environmental constraints and is not enough to control pathogens under field conditions. Instead, the combination of these vital strategies with fungicides can in turn lead

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to the integrated management of the disease. Since fungi can remain as mycelium or clamydospores in seeds and soil, as well as on the infected crop residues buried in the soil for up to six years [5], and the early wilting causes more loss than late wilting [6], seed treatment has been provided as the easiest control method, since the appropriate seed dresser can control pathogens transmitted by seeds, also it has a protective effect against soil-borne fungi [16].

This work aimed to evaluate some seed dressers to control Fusarium wilt disease on chickpea during the germination and seedling stages under glass house conditions.

2. Materials and Methods

2.1 Fungal Isolates

This work was conducted at the National Commission for Biotechnology, and the isolated *Fusarium oxysporum* f. sp. *Ciceri* was obtained from the Plant Pathology Laboratory in the Commission, it was isolated from the roots of infected chickpea plants cultivated in Gab area, Syria during 2018. The fungal isolate was cultured in Petri dishes on PDA medium, and kept in the fridge until use.

2.2 Chickpea Seed: Chickpea cv. Ghab3

2.3 Treatment of Seeds with Fungicides

The seeds were treated with the tested seed dressers as recommended by the manufacturer (Table 1). The seeds were soaked for 2 minutes in the pesticide solutions, while the control seeds were soaked in distilled water only.

2.4 Artificial Infection and Pesticide Testing

The treated seeds, either with the three tested pesticides or distilled water, were planted in plastic trays, and the infection was carried out by placing a piece of agar containing the fungal mycelium next to the seed. Each treatment (three pesticides, a negative control; infected untreated treatment and a positive control; uninfected untreated treatment) had 24 seeds. For each treatment, seed germination and seedling mortality were evaluated after 15 and 30 days of cultivation, respectively.

2.5 Statistical Analysis

The statistical analysis was done using one-way ANOVA at a significant level of 5%, by SPSS version 14.

3. Results and Discussion

Chickpea seeds were treated with three fungicides, and infected with *Fusarium oxysporum* f. sp. *Ciceri* after planting directly. The percents of both seed germination and seedling mortality were evaluated after 15 and 30 days of planting, respectively.

The results showed that the percent germination in the negative control (C-) was 91.76%, with a significant difference to the positive control (C+) 37.5% (Fig. 1), which was attributed to that Fusarium infects seedling after 3 to 5 days of planting, causing the death and flattening on the ground [6].

The treatment of seeds with fungicides enhanced the germination. Thus, the treatment with Vitaflo increased the percent germination significantly compared with C+, but did not exceed 58.44%. While both of OxyCure and Topsin were the superiors with a percent germination reaching 79.17%, 75% respectively. This revealed that the treatment of chickpea seeds with fungicides reduced the incidence of Fusarium in faction significantly and the percent

Table 1 The commercial names of the tested pesticides and percentage of active ingredient and rate of use of each.

Fungicide	% Active ingredient	Rate of use
Oxycure-wp	85% Dicopperchloridetrihydroxyde	500 g/100 L water
Topsin M	70% Thiophanate-methyle	50 g/100 L water
Vitaflo 280 FS	15% Carboxin + 13% Thiram	3-3.5 L/ton

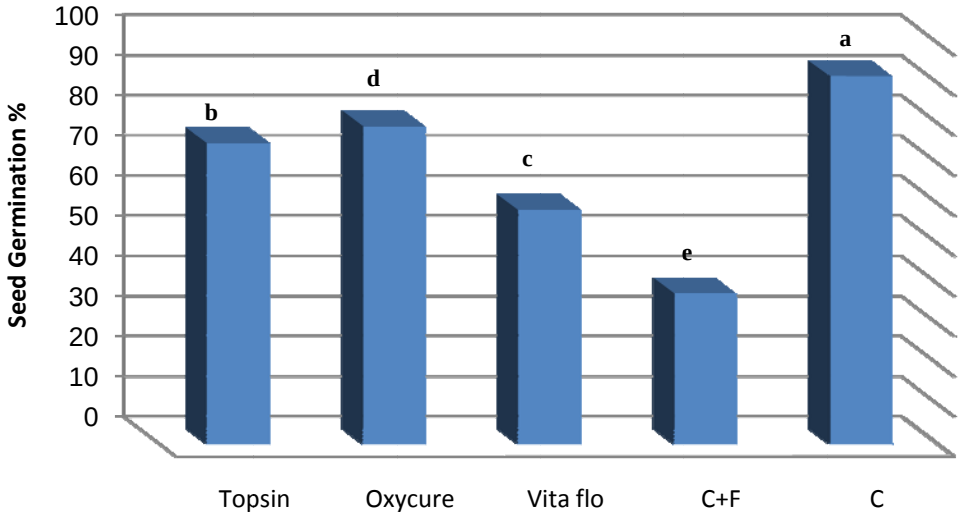

Fig. 1 Effect of chickpea seed treatment with the tested fungicides on the germination after *Fusarium oxysporum* f. sp. *Ciceri* infection.
The different letters above the shapes indicate significant differences ($p < 0.001$) among treatments (ANOVA followed by LSD).

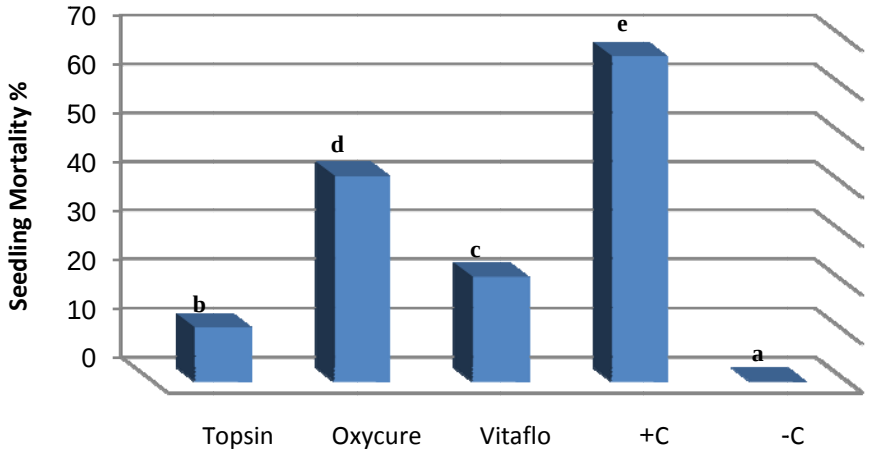

Fig. 2 Seedling mortality of different treatments after 30 days of planting.
The different letters above the shapes indicate significant differences ($p < 0.001$) among treatments (ANOVA followed by LSD).

germination varied depending on the fungicide [17]. In addition, the seed dressers protect them from several soil fungi like *Fusarium* sp. [18].

The results recorded, after 30 days of planting, were shown in Fig. 2.

For the positive control C+, the percent of seedling mortality was 66.67%, which was characterized by small leaves, pale color, and stopping growth led to the fall after a while, whereas the plants showed no change in leaf size, and there was no seedling fall after emerging above the soil, in the negative control C-. That was related to the physiological changes in the infected leaves [19], causing yellowing then wilting [20], as that the infected seedlings get pale green and weaken, and finally collapse [21]. Since the fungus penetrates the vascular system in the root, by producing an enzyme breaks the cell wall, which forms a gel plugs the canals and inhibits passage of the sap, leading to the discoloring and flattening to the ground [22, 23].

For Vitaflo, the percent of seedlings mortality decreased to 21.43%, by a significant difference with

C+, as Thiram can inhibit fungal growth up to 78.14%, *in vitro* [24]. While the infected seedlings fell down, the rest did not show any symptoms, which can be attributed to the role of Carboxin in promoting plant growth by increasing the content of chlorophyll in leaves [25]. Controversy, the percent of seedlings mortality increased to 42.1% for OxyCure, with significant differences with all other treatments, and the seedlings were weak with small-sized and pale color leaves, and then collapsed. Among all treatments, Topsin was more effective than other seed dresser fungicides and showed the least seedling mortality (11.11%). This pointed to the efficient inhibition of Topsin and its derivatives against *Fusarium oxysporum* f. sp. *Ciceri* [26-28], since using Topsin as a seed dresser could cause less than 6% of dead seedlings [29]. On the other hand, Thiram and Carbendazim (Topsin derivative) have shown a good efficacy as seed dressers in controlling Fusarium wilt [30] and had superiority over the fungicide OxyCure *in vitro* [31].

It is noted that both of copper compound; OxyCure, and Topsin achieved the highest percent of germination in comparison with that of Vitaflo. While there was an increase in seedling mortality for OxyCure, Topsin provided better protection to the plants. This can be explained by that copper compound was able to protect the seeds from fungus during the germination phase, thus the percent of germination was relatively high, but it could not keep its efficacy to protect the seedlings; as it is a contact fungicide. Nevertheless, Topsin, which has a systemic action, appeared to protect seedling even after emerging above the soil, so it is a preventive and curative fungicide [32]. Moreover, the copper compound has a lower efficiency in controlling Fusarium wilt [32], in addition to its toxicity to plants which caused increase of seedling mortality, as that the difference between the fungi toxic and the phototoxic concentrations of copper is small, it was shown that seed treatments with copper compound often weaken the germination capacity of seeds like wheat [32]. It should be noted here, that the active ingredient in Topsin; Thiophanate-methyle is not effective by itself [33], but it is metabolized in plant cells to benzimidazole derivatives; the active ingredient [34-36]. Moreover, in fungi cells, it can be metabolized to Carbendazim, which is a fungicide as it is one of benzimidazole derivatives [37].

The current work showed that Vitaflo was the least effective fungicide used in *Fusarium oxysporum* f. sp. *Ciceri* to control at the germination stage, but it went on better than OxyCure at seedlings stage. Vitaflo contains two effective elements: the systematic element (Carboxin) provides it with the antifungal ability to protect the seedlings, and the contact one (Thiram) is effective against soil-borne infections and provides protection during germination stage, but it is less effective than OxyCure.

4. Conclusions

Fungicides applied as seed dressers reduced disease incidence significantly, and the effectiveness of seed dressers in controlling *Fusarium oxysporum* f. sp. *Ciceri*, which infects chickpea as, was different depending on the fungicide used.

Seed coating with Topsin can increase the germination percent and protect seedlings simultaneously. Although it has a systemic mode of action only, it has an efficiency not just less than the copper compound (contact fungicide) but is better than that of Vitaflo (contact and systematic fungicide).

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