Suppressive effect of commercial disinfectant and sterilized agents formulated as a dustable powder against Fusarium wilt in bean plants

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Abstract:

Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *phaseoli* (FOP), leads to notable declines in the yield and quality of common bean plants. Globally, there is a tendency to evaluate the efficacy of substances against phytopathogenic fungi as an alternative to pesticides. Thus, this study aims to assess the efficacy of disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a dustable powder (DP 20%) against FOP both in *vitro* and in *vivo*. The *in vitro* antifungal activity was conducted using the poisoned food technique with five concentrations of 200, 400, 600, 800, and 1000 ppm. Formulated disinfectant agents were tested *in vivo* by seeds dressing for common bean (Giza 6) in three concentrations of 0.4, 0.8, and 1.2% before sowing in soil infested by FOP compared with Rizolex-T 50% WP at the recommended dose (3g Kg^{-1} seeds). Growth parameters (Fresh and dry weight, shoot and root length), chemical constituents, and enzyme activities (catalase and peroxidase) of bean plants infected by FOP were investigated at 45 days post-sowing. Findings proved that chloroxylenol exhibited more efficacy against FOP *in vitro* compared to the other tested substances, as EC_{50} was 351.15mg/L, whereas phenol and phenic had EC_{50} of 628.14 and 832.39mg/L, respectively. *In vivo*, the application of disinfectant agents formulated as a dustable powder (DP, 20%) and Rizolex-T 50% resulted in a significant reduction in disease incidence, as well as an increase in growth parameters. The intervention resulted in a substantial increase in both total soluble sugars and total chlorophylls. Furthermore, it resulted in a decrease in proline levels and enzyme activities. The highest reduction in disease incidence as well as an increase in growth parameters and chemical compositions were recorded in seedlings treated with chloroxylenol at concentrations of 0.8 and 1.2%, followed by phenol at 1.2%, whereas phenic was found to have the least effectiveness. Therefore, chloroxylenol formulated as DP 20% exhibits promise in controlling fusarium wilt disease and enhancing bean plant's yield and quality, also safe and less expensive compared to fungicides.

Keywords: *Fusarium oxysporum* f.sp *phaseoli*; chloroxylenol; phenic; phenol; antifungal effect; bean plants

1. Introduction:

The common bean (*Phaseolus vulgaris L.*) belongs to the legume family. It is the most economically paramount leguminous crop in Egypt and all over the world **(Abdel-Hakim *et al*., 2012)**. Beans contain carbohydrates, proteins, vitamins, minerals, and antioxidants. Additionally, they are rich in unsaturated fatty acids, specifically linoleic and oleic acids **(Celmeli *et al*., 2018)**.

Fusarium oxysporum f. sp *phaseoli* is a soil-born plant pathogen in the phylum Ascomycota, Family Nectriaceae **(Kendrick and Syder, 1942)**. It is the causal agent of fusarium wilt disease of bean plants. It exhibits long-term persistence in soil and leads to significant reductions in yield and quality, especially in developing countries. It can cause a 100% yield loss in bean production **(Muriungi *et al*., 2013)**. Wilted plants exhibit leaf yellowing and wilting, which progressively infiltrates the younger leaves, resulting in the weakening of the plants. The newly formed leaves turn

yellow and wilt while older leaves droop **(Dubey, 2014)**.

The control of soil-born plant diseases is intricate due to their residence in or near the dynamic rhizosphere environment and their ability to persist in soil for extended periods by developing resistant survival structures **(El-Mougy *et al*., 2012)**. Currently, fungicides are the most efficient approach for controlling wilt fungi, also it can persist in the soil for many years. Furthermore, the extensive application of fungicides has led to the development of fungicides resistance around the world **(Alam *et al*., 2010)**.

Disinfectants are incapable of killing all microorganisms in certain mediums, but they can effectively diminish their population to a minimum harmful scale. The efficiency of these compounds varies based on their constituents **(El-Mougy *et al*., 2020)**. Disinfectant agents consisting of chemical active agents can inhibit the non-sporing or vegetative growth state of pathogenic microorganisms. They are utilized on the surfaces of instruments and healthcare objects to control and prevent infection **(Rutala, 1995)**. They can destroy the cellular structure of microbes or impede their metabolic processes. Additionally, they interact with the cell surface penetrating the cell and acting on the target sites **(Russell and Chopra, 1996)**. The microbial inhibition is supposed to be caused by phenolic compounds, which sensitize the phospholipid bilayer of the cytoplasmic membrane, resulting in reduced permeability and inaccessibility of vital intracellular components **(Juven *et al*., 1994)**.

Dettol is composed of chloroxylenol B.PC (4.8% w/v). Its antimicrobial effect is attributed to its phenolic properties, which disrupt the permeability of the microorganism's cell wall, hence inhibiting the activity of enzymes, and ultimately cell death **(Kim *et al*., 2002)**. Chloroxylenol (Dettol) is a broad-spectrum antimicrobial agent extensively used in industrial, consumer, and healthcare products, including cosmetics, household chemicals, and disinfection products such as preservatives or disinfectants **(Poger and Mark, 2019)**. Furthermore, it strongly affects Gram-positive bacteria, as it strongly affects *Staphylococcus aureus* spp **(Al-Beshari *et al*., 2018)**. Furthermore, chloroxylenol acts on the cytoplasmic membrane of bacteria, causing protein denaturation. **Papageorgiou and Chu (2000)** noted that chloroxylenol is non-irritating bactericidal against most Gram-positive bacteria, but it is less effective against Gram-negative bacteria. Therefore, to kill all cells completely, the concentration must be high enough **(El Mahmood and Doughari, 2008)**. The antimicrobial properties of disinfectant agents against some pathogenic bacteria have been reported **(Mellefont et al., 2003; El Mahmood and Doughari, 2008)**.

To offer a low-cost, safe, and effective method of pest management, pesticide formulation is the process of changing a pesticide into a form that can be conveniently made, stored, moved, administered to change it into a product that can be stored, moved, and used in real-world scenarios to produce outcomes that are efficient, safe, and economical **(Hazra and Purkait, 2019)**. Therefore, current study aimed to investigate the efficacy of disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a dustable powder (DP 20%) against *Fusarium oxysporum*-infected bean plants in both *vitro* and in *vivo*.

2. Materials and methods:

2.1. Tested materials

2.1.1. Commercial Disinfectants

a. Chloroxylenol, or para-chloro-meta-xylenol (PCMX), is a mixture of 4.8% chloroxylenol + 9.9% terpineol and absolute alcohol. It was supplied by Agricultural Development Markets, Nadi El Seid St., Dokki, Giza.

b. Phenic contains more than 98% saponified tar oils and carbonates. It has between 6.5 and 7% pure phenol. It is produced by the International Company for Chemicals and Industrial Detergents (Cairo, Egypt).

2.1.2. Active ingredient

Phenol or carbolic acid (C_6H_5OH): A white volatile crystalline solid, was supplied by EL-Gomhoria Co., Cairo, Egypt.

2.1.3. Diluent material

Talc powder (magnesium meta silicate): Talc is a mineral composed of hydrated magnesium silicate with the chemical formula $H_2Mg_3(SiO_3)_4$ or $Mg_3Si_4O_{10}(OH)_2$, it is the widely used substance known as talcum powder, was supplied by El-Nasr Co. for Phosphate, Cairo, Egypt.

2.1.4. Solvents

Absolute ethanol, acetone, and xylene were supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2. The physico-chemical properties of basic formulation components

2.2.1. Active ingredient

a) Solubility of active ingredient was determined by measuring the volume of distilled water, acetone, and xylene for complete solubility of one gram at 20°C **(Nelson and Fiero, 1954)**. The

solubility (%) was calculated using the following equation:

$$\text{Solubility (\%)} = \frac{W}{V} \times 100 \quad (1)$$

Where W= weight of active ingredient, and V= volume of solvent required for full solubility.

b) Free acidity or alkalinity was determined as per the method outlined in **FAO/WHO MT 191 (2010)**.

2.2.2.The physico-chemical properties of diluent

a) Free acidity or alkalinity was determined as mentioned before.
b) Dry sieve test was conducted in accordance with **(CIPAC, MT 59.1, 2002)**.
c) Bulk density was determined using **FAO/WHO MT 186 (2010)**.

2.3. Preparation of commercial disinfectants as a dustable powder (DP)

The dustable powder (DP, 20%) formulation was prepared using the dry mix method (Furmidge, 1972). It comprises 20% active ingredient (wt/wt) or commercial disinfectants combined with a suitable diluent of 80%. The combination is well mixed and passed through a 74-micron sieve **(El-Sisi, 1986)**.

2.4. Determination of the physico-chemical properties of the new locally prepared dustable powder (DP 20%) formulation

a) Free acidity or alkalinity was determined as mentioned before.
b) Dry sieve test was determined as mentioned before.
c) Bulk density was determined as mentioned before.

2.5. Isolation and identification of the fungal pathogen

Bean plants exhibiting characteristic symptoms of fusarium wilt were collected from a private field in Qaliubia Governorate, Egypt. Samples showing different symptoms ranging from foliar chlorosis, necrosis, stunting, wilting, vascular discoloration, stem necrosis, and death were collected. The infected portions were rinsed with tap water to remove the adhering soil particles, then cut into small fragments and sterilized by immersing them in a 5% sodium hypochlorite solution for 5 min **(Burr *et al*., 1978)**. The segments underwent multiple washes using sterilized distilled water, followed by drying between two layers of sterilized filter papers. Subsequently, the segments were transferred to sterilized petri dishes containing potato dextrose agar medium (PDA). Subsequently, plates were incubated at 25±2°C, and developed colonies were picked up after seven days, transferred into a new PDA medium, and purified using hyphal tip techniques **(Dhingra and Sinclair, 1984)**. The isolated fungi was identified microscopically **(Barnett and Hunter, 1987)**. The identification was conducted at Plant Pathology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. The stock culture was preserved on PDA slants and maintained at 10°C.

2.6. Antifungal assay *in vitro*

The antifungal activity of disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a dustable powder (PD 20%) against *Fusarium oxysporum* was determined by food-poisoned technique **(Mohanty *et al*., 2012)**. Disinfectant agents formulated at 200, 400, 600, 800, and 1000 ppm were mixed with 50ml of sterilized PDA medium and transferred equally into three petri dishes. The media was allowed to solidify. Then seven-day old fungal culture disk of 9mm diameter was taken and inoculated to the center of petri dishes containing disinfectant agents. A PDA medium without disinfectant agents served as control. All dishes were incubated at 25±2°C and radial growth of colony was measured when the mycelia of control had almost filled the petri dishes. Each test was performed in triplicate. The fungal growth inhibition was calculated due to treatments against control using the following formula **(Rai *et al*., 2014)**.

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100 \quad (2)$$

Where C is the average of three replicates of hyphal extension (mm) of control and T is the average of three replicates of hyphal extension (mm) of plates treated with tested material. EC_{50} and EC_{90} values were determined by the linear regression (LPD) line computer program) of the probit of the tested fungus percentage inhibition vs. Logs the concentrations (mg/l) of the disinfectant agents. The EC_{50} and EC_{90} notation is used to indicate the effective concentrations (mg/l) that cause 50% and 90% growth inhibition, respectively. In essence, the lower the value of EC_{50} and EC_{90} the higher the efficacy of disinfectant agents in the test under consideration.

2.7. Evaluation of different formulated disinfectant agents in controlling

Fusarium oxysporum f. sp. *phaseoli in vivo*.

The efficacy of disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a dustable powder 20% against *Fusarium oxysporum* f.sp. *phaseoli* (FOP) compared with Rizolex-T 50% WP (tolclofos-methyl 20% + thiram 30%, Sumitomo Japan) were estimated under greenhouse conditions [temperature (25°C±2°C) and humidity (65%)] at the Central Agriculture Pesticide Laboratory, ARC, Giza, during the growing season 2022. The sandy clay soil was sterilized using a formalin solution (5%) for 24 hrs and left for a weak to get rid of formalin. Pots were filled with cornmeal sand medium at 3% (W/W), infested by FOP, and watered regularly for 10 days before sowing to ensure the distribution of inoculum. Common bean seeds (Giza 6) coated as seed dressing with the tested materials at different concentrations of 0.4, 0.8, and 1.2% and Rizolex-T 50% at the recommended dose of 3g Kg^{-1} seeds, individually **(El-Mougy *et al*., 2007)**. Bean seeds were sowed at the rate of five seeds/pot and four replicates (pots) were used for each treatment. The percentage of wilt incidence of beans at pre-and post-emergence stages was estimated after 15 and up to 45 days of the experimental period **(Cachinero *et al*., 2002)** as follows:

$$\text{Pre-emergence damping off} = \frac{\text{number of un-emerged seedlings} \times 100}{\text{number of sown seeds}} \quad (3)$$

$$\text{Post-emergence damping off} = \frac{\text{number of dead seedlings} \times 100}{\text{number of sown seeds}} \quad (4)$$

$$\text{Survival plants} = \frac{\text{number of survived plants} \times 100}{\text{number of sown seeds}} \quad (5)$$

2.8. Growth parameters, chemical constituents, and enzyme activities of bean plants

2.8.1. Growth parameters: Shoot fresh and dry weight, and shoot and root length of bean plants infected by FOP were determined after 45 days post-sowing.

2.8.2. Total soluble sugars (TSS) were determined according to the Shaffer-Somogi micro method and expressed as glucose **(A.O.A.C., 1995)**.

2.8.3. Total Chlorophyll was extracted by Dimethyl sulphoxide (DMSO) according to **(Villanueva *et al*., 1985)**. The optical densities were measured spectrophotometrically at 662, 644, and 470 nm. The concentration was calculated using Wettestein's formula in **(A.O.A.C., 1995)**.

2.8.4. Proline was extracted using sulfosalicylic acid 3% (w/v) and determined according to **(Bates *et al*., 1973)**.

2.8.5. Antioxidant enzyme activities were determined according to **(Bradford, 1967)**. Leaf tissues were homogenized in 100Mm chilled sodium phosphate buffer (pH 7) at 1:4 (w/v). The supernatant was used to measure the activities of catalase **(Aebi, 1984)** and peroxidase activities **(Hammerschmidt *et al*., 1982)**.

2.9. Statistical analysis

The obtained data were subject to analysis of variance (ANOVA), using Minitab Statistical Software 20 **(Gomez and Gomez, 1984)**. Significant differences between means were compared at $p < 0.05$ following Tukey's post hoc test.

3. Results:

3.1. Formulation part

Table 1 displays the physico-chemical properties of phenol as an active ingredient. Phenol has shown acidic properties (free acidity 0.098) and displayed full solubility in acetone (100%) and moderate solubility in water and xylene (62.5%).

Table 1. Physico-chemical properties of phenol as an active ingredient

Solubility % (W/V)			Free acidity as
Water	**Acetone**	**Xylene**	**%H_2SO_4**
33	100	62.5	0.098

Table 2 shows the physico-chemical properties of the suggested diluent (talc) to determine if it was suitable to prepare the tested materials as dustable powder formulations. According to the dry sieve test, tested diluent passed successively (100%) through the 74µ sieve. Furthermore, the test revealed that talc had a minor acidity, with a free acidity percentage of 0.0196. The bulk density of the diluent before and after compaction was measured to be 0.36 and 0.41, respectively. These suggest that the fluctuation percentage in bulk density between the pre-compaction and post-compaction should not exceed 60%. The selection of a suitable diluent is of utmost importance in the formulation of dry insecticides, as it significantly impacts the chemical stability of the introduced toxicants. Ideally, pesticide formulators

should possess knowledge regarding the characteristics of the diluent before its selection.

Table 2. Physico-chemical properties of diluent used for DP 20% formulation

Diluent	Free acidity % as H_2SO_4	Dry sieve test (% passed through 74micron sieve)	Bulk density	
			Before	After
Talc	0.0196	100	0.36	0.41

The data presented in Table (3) demonstrate that all tested dustable powder passed successfully through a 74-micron sieve, following the recommendations of the **FAO/WHO (2002)**. In contrast, it was seen that chloroxylenol and phenic had minor alkaline characteristics (measured as sodium hydroxide), whereas phenol demonstrated a weak acidic characteristic (measured as sulfuric acid). Furthermore, the three formulations adhered to the guidelines of **(W.H.O. 1979)** regarding bulk density both before and after compaction. The bulk density (%) pre- and post-compaction should not exceed 60% of the density pre-compaction.

Table 3. Physico-chemical properties of disinfectant agents formulated as DP 20%

Compounds	Free acidity % as H_2SO_4	Free alkalinity % as NaOH	Dry sieve test through 74 µ	Bulk density	
				Before	After
Phenol	0.098	-	passed	0.40	0.80
Chloroxylenol	-	0.96	passed	0.38	0.64
Phenic	-	0.24	passed	0.42	0.63

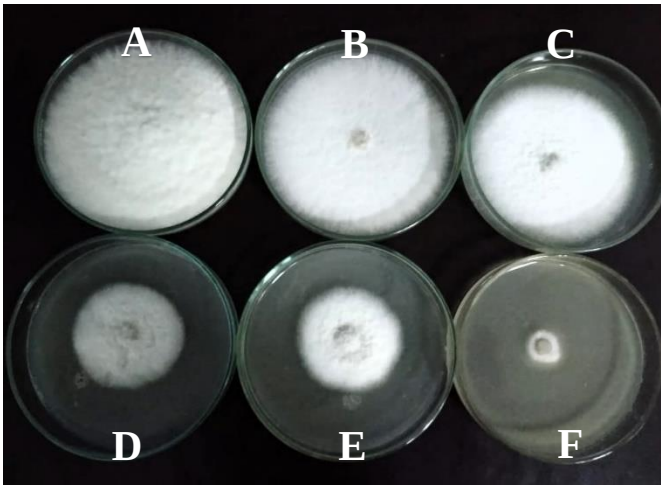
3.2. The inhibitory effect of the disinfectant agents formulated as DP 20%

Results depicted in Table (4) and Figure (1) show the inhibitory effect of the disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a 20% dustable powder against *F. oxysporium*. Linear growth of *F. oxysporum* significantly decreased with increasing the concentration of the disinfectant agents. Chloroxylenol had the highest level of efficiency among all tested disinfectant agents. At 200 ppm, the chloroxylenol formulation showed a 32.2% inhibition against *F. oxysporum*, whereas phenol and phenic formulations did not exhibit any efficacy. Furthermore, it attained the minimum EC_{50} value of 351.15 ppm, whereas EC_{50} values for phenol and phenic were 628.14 and 832.39 ppm, respectively. The inhibitory effect of the chloroxylenol formulation may be attributed to the presence of chloroxylenol 4.8% and terpineol 9.9% which have an inhibitory effect against microorganisms. Despite phenol at a concentration of 200 ppm was less effective, it achieved the highest inhibition of fungal growth at a higher concentration of 1000 ppm, which reached 90.0%. The disinfectant agents exhibited inhibitory effects ranging from 0.0 to 90.0% for phenol, 32.2 to 82.6% for chloroxylenol, and 0.0 to 63.7% for phenic.

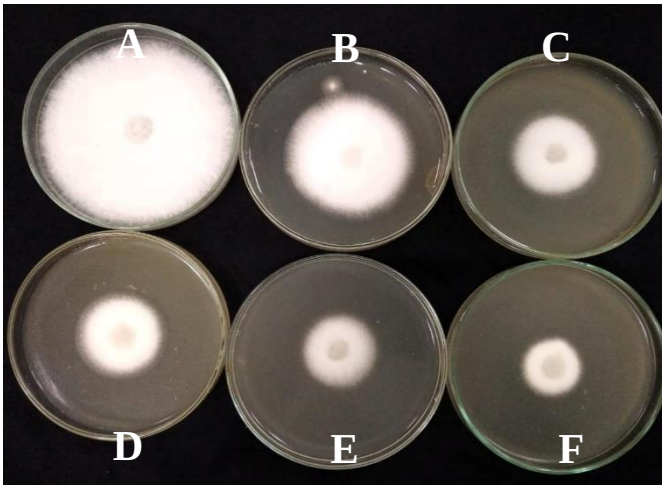
Table 4. The inhibitory effect of the disinfectant agents formulated as DP 20% against FOP

Compounds	Concentrations (ppm)					EC_{50} *	EC_{90}	Slope value
	200	400	600	800	1000			
Phenol	0.0	20.3	44.1	60.0	90.0	628.14	1171.82	4.7326+/-0.4093
Chloroxylenol	32.2	54.4	66.3	74.8	82.6	351.15	1603.02	1.9435+/-0.2441
Phenic	0.0	21.1	30.3	42.4	63.7	832.39	2108.40	3.1752+/-0.3464

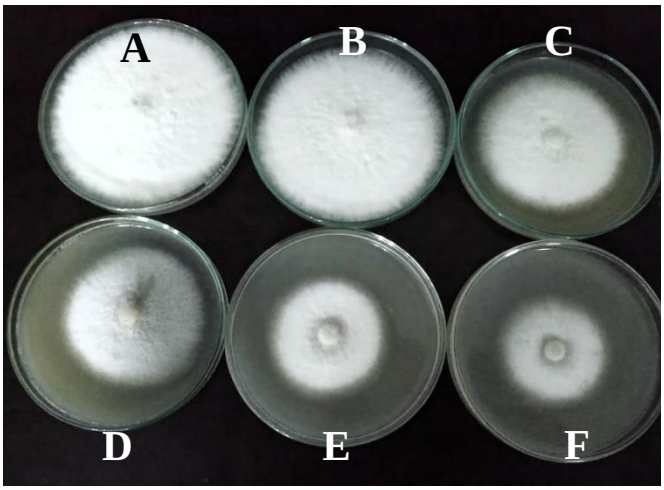
* ppm; parts per million, EC_{50} and EC_{90} indicate the effective concentrations (mg L^{-1}) that cause 50% and 90% growth inhibition, respectively.



a. Phenol



b. Chloroxylenol

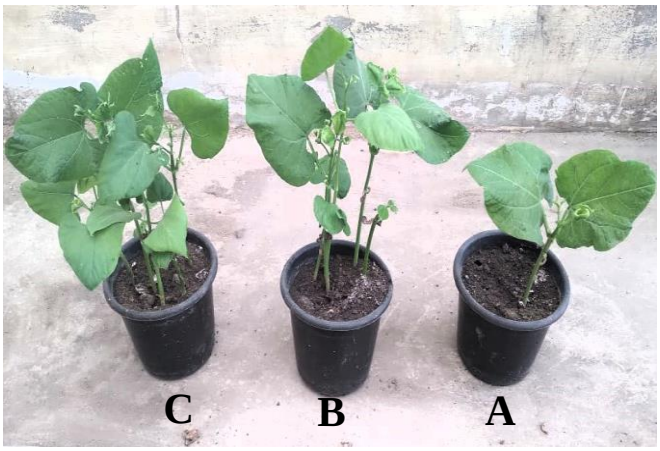


c. Phenic

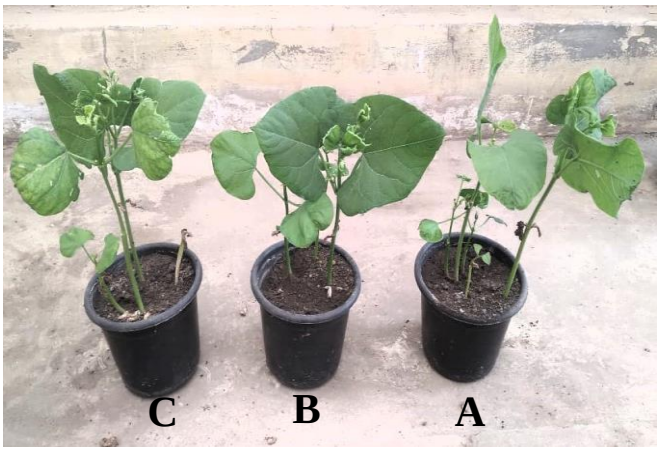
Fig. 1 (a-c). The suppressive effect of disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a dustable powder (20%) against *Fusarium oxysporum* f. sp. *phaseoli*. A. Control, B. 200 ppm, C. 400 ppm, D. 600 ppm, E. 800 ppm, and F. 1000 ppm.

3.3. Evaluation of disinfectant agents formulated as DP 20%, and Rhizolex-T on disease incidence

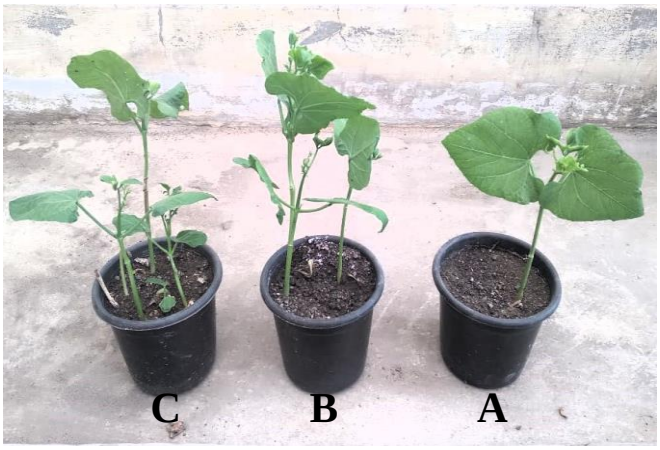
During the pre-emergence stage, the presence of FOP resulted in a 60% inhibition of seed germination. The germination rate was reduced to 25%, with only 15% of seedlings surviving (Figure 2 a-d). Treating seeds with disinfectant agents' formulation and Rizolex-T 50%, effectively suppressed the incidence of fusarium wilt disease compared to the control infected soil under greenhouse conditions. Data in Table (5) indicate that Rhizolex-T, phenol at a concentration of 1.2%, and chloroxylenol at a concentration of 1.2% exhibited the most significant decrease in pre- and post-emergence damping off. This was expressed in a higher percentage of survival plants (85%), while the lowest one was attributed to phenic at 0.4%.



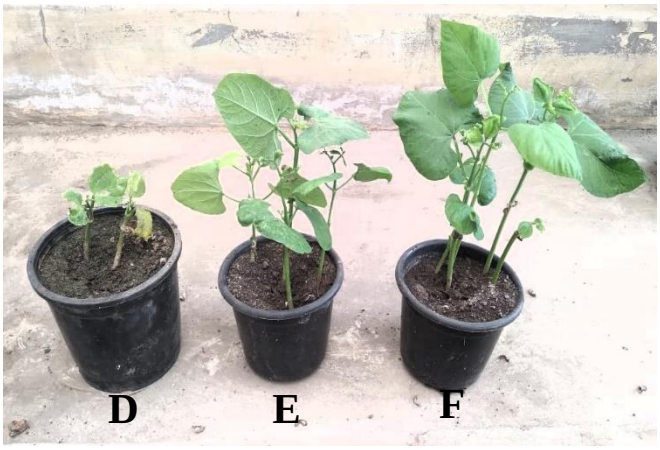
a. Phenol



b. Chloroxylenol



c. Phenic



d. Rizolex-T 50%

Figure 2 (a-d). Effect of disinfectant agents (phenol, chloroxylenol, and phenic) formulated as a dustable powder (20%), and Rhizolex-T on disease incidence. A; conc 0.4 %, B; conc 0.8%, C; conc 1.2%, D; control pathogen, E; fungicide, and F; control healthy.

Table 5. Effect of disinfectant agents formulated as DP 20% on disease incidence *in vivo*.

Treatments	Pre-emergence (%)	Post-emergence (%)	Survived plants (%)
Control infected soil	60	25	15
Control sterilized soil	0	0	100
Phenol at 0.4%	20	15	65
Phenol at 0.8%	10	10	80
Phenol at 1.2%	10	5	85
Chloroxylenol at 0.4%	25	10	65
Chloroxylenol at 0.8%	20	10	70
Chloroxylenol at 1.2%	10	5	85
Phenic at 0.4%	30	15	55
Phenic at 0.8%	20	15	65
Phenic at 1.2%	20	10	70
Rizolex-T 50%	10	5	85

3.4. Effect of disinfectant agents formulated as DP 20% on growth parameters of bean seedlings infected by FOP

Disinfectant agents (Phenol, Chloroxylenol, and Phenic) formulation at different concentrations and Rizolex-T 50% significantly influenced the growth parameters of bean plants compared to those grown in infected soil (Table 6). Bean plants grown in infected soil recorded a reduction in shoot and root length

compared to those grown either in sterilized soil or in soil treated with disinfectant agents' formulation at different concentrations and Rizolex-T 50%. Sowing bean seeds in sterilized soil greatly increased shoot and root length by 107% and 85%, respectively compared with those sown in infected soil. The increase in shoot and root lengths for bean seedlings was observed after 45 days of sowing seeds treated with all treatments. This effect was noticeable in the case of treatment with chloroxylenol (1.2%), which reached 128% and 104%, respectively compared with plants grown in infected soil. Similarly, the same observation was shown in the case of total length.

Additionally, sowing bean seeds in infected soil caused a great reduction in shoot fresh and dry weights compared with those sown either in sterilized soil or in soil treated with disinfectant agents' formulation at different concentrations and Rizolex-T 50%. The improvement in fresh and dry weights for bean seedlings was observed at 45 days after sowing the seeds treated with all treatments. This effect was more pronounced with chloroxylenol at a concentration of 1.2%. Compared with plants grown in infected soil, chloroxylenol 1.2% increased fresh and dry weight of bean plants by 161% and 127%, respectively.

Table (6) Effect of disinfectant agents formulated as DP 20% on growth parameters of bean seedlings infected by FOP

Treatments *	Shoot length (cm)	Root length (cm)	Total length (cm)	Fresh weight (g)	Dry weight (g)
Control infected soil	15.3±0.3^{e}	8.7±0.7^{d}	24.0±0.6^{i}	3.66±0.21^{e}	0.44±0.02^{f}
Control sterilized soil	31.7±1.7^{ab}	16.0±0.6^{ab}	47.7±2.2^{a-d}	8.26±1.09^{a-c}	1.02±0.05^{a}
Phenol at 0.4%	24.7±0.3^{cd}	14.7±2.0^{ab}	39.3±1.8^{fg}	7.01±0.34^{b-c}	0.71±0.06^{de}
Phenol at 0.8%	25.7±0.9^{c}	17.0±0.6^{a}	42.7±1.3^{d-g}	8.40±0.76^{a-c}	0.79±0.01^{b-d}
Phenol at 1.2%	32.3±1.5^{a}	17.3±0.9^{a}	49.7±2.3^{a-c}	9.48±0.25^{a}	0.89±0.04^{a-c}
Chloroxylenol at 0.4%	31.3±2.9^{ab}	15.3±0.9^{ab}	46.7±2.3^{b-e}	8.66±0.35^{ab}	0.82±0.06^{b-d}
Chloroxylenol at 0.8%	33.8±2.0^{a}	17.0±0.6^{a}	50.8±2.5^{ab}	9.25±0.16^{a}	0.92±0.01^{ab}
Chloroxylenol at 1.2%	35.0±1.3^{a}	17.7±0.7^{a}	52.7±1.8^{a}	9.56±0.68^{a}	1.00±0.05^{a}
Phenic at 0.4%	21.2±2.0^{d}	10.0±1.2^{cd}	31.2±3.1^{h}	6.77±0.13^{cd}	0.61±0.06^{e}
Phenic at 0.8%	27.3±1.5^{bc}	14.3±2.0^{ab}	41.7±1.8^{e-g}	6.91±0.97^{b-c}	0.77±0.03^{cd}
Phenic at 1.2%	27.7±0.7^{bc}	16.7±0.9^{ab}	44.3±1.4^{c-f}	7.88±0.91^{a-d}	0.82±0.05^{b-d}
Rizolex-T 50%	25.0±0.6^{cd}	13.3±1.8^{bc}	38.3±2.2^{g}	6.27±0.24^{d}	0.79±0.07^{b-d}
p-value	*	*	*	*	*

* Values are means ± standard error (n = 3). * and ** indicate differences at $p \leq 0.05$ and 0.01 probability level following Tukey's post hoc test.

Table 7 represents the data on the analysis of variance (ANOVA) on total chlorophyll, total soluble sugars, and proline content of bean plants grown under different concentrations of disinfectant and Rizolex-T 50% at p <0.05, and <0.01. Bean plants grown in sterilized soil recorded an increase in total chlorophyll (T.Chl.) and total soluble sugars (TSS) by 45% and 39%, respectively compared with those sown in infected soil. Using the disinfectant and Rizolex-T 50% improved T.Chl. and TSS with a great increment of 45% and 28% respectively were recorded in plants treated with chloroxylenol 1.2% compared with non-treated one. Additionally, plants grown in infected soil recorded higher levels of proline (8.67 µmole g^{-1} DW), indicating that these plants suffered from biotic stress, compared with those grown either in sterilized soil or in soil treated with disinfectant agents' formulation at different concentrations and fungicide. Plants treated with disinfectant formulated and Rizolex-T 50%, caused a reduction in proline content. The reduction was highly pronounced in the case of seeds grown in sterilized soil by 44%, also the seeds treated with Rizolex-T 50% and chloroxylenol 1.2% by 38%, followed by chloroxylenol 0.8% which recorded a 33% reduction in proline content.

Table (7). Effect of disinfectant agents formulated as DP 20% on total soluble sugars (TSS), proline, and total chlorophyll of bean seedlings infected by FOP

Treatments	Total chlorophyll (mg g^{-1} FW)	Total Soluble Sugars (mg g^{-1} FW)	Proline content (µg g^{-1} FW)
Control infected soil	2.5±0.20^{d}	10.8±0.21^{g}	8.7±0.26^{a}
Control sterilized soil	3.6±0.23^{a}	15.0±0.33^{a}	4.9±0.20^{g}
Phenol at 0.4%	2.9±0.10^{b-d}	11.6±0.23^{e-g}	7.5±0.20^{b}
Phenol at 0.8%	2.9±0.18^{cd}	11.8±0.38^{ef}	6.5±0.21^{c}
Phenol at 1.2%	3.1±0.06^{a-c}	12.4±0.43^{c-e}	6.2±0.17^{c-e}

Chloroxylenol at 0.4%	3.0±0.09^{a-c}	12.8±0.09^{cd}	6.4±0.21^{c-e}
Chloroxylenol at 0.8%	3.4±0.17^{ab}	13.2±0.30^{bc}	5.8±0.20^{ef}
Chloroxylenol at 1.2%	3.6±0.20^{a}	13.8±0.49^{b}	5.4±0.09^{fg}
Phenic at 0.4%	2.8±0.12^{cd}	11.2±0.28^{fg}	6.6±0.35^{cd}
Phenic at 0.8%	2.8±0.10^{cd}	12.0±0.17^{d-f}	6.4±0.34^{c-e}
Phenic at 1.2%	2.9±0.20^{b-d}	12.3±0.20^{c-e}	6.0±0.15^{d-f}
Rizolex-T 50%	3.2±0.35^{a-c}	13.0±0.15^{c}	5.4±0.12^{fg}
p-value	**	*	*

* Values are means ± standard error (n = 3). * and ** indicate differences at $p \leq 0.05$ and 0.01 probability level following Tukey's post hoc test.

Antioxidant enzyme activities in bean plants were significantly affected by the application of Rizolex-T 50%, and disinfectant agents at different concentrations (Table 8). Data illustrates that sowing bean seeds in soil infested with FOP increases the activities of catalase (CAT) and peroxidase (POD) enzymes. This increase was more than 2-fold higher in plants cultivated in infested soil than in control sterilized soil. The application of Rizolex-T 50% and disinfectant agents at different concentrations caused a decrease in CAT and POD activities compared with control-infested soil. The reduction percentage in activities of CAT and POD was more noticeable in plants treated with Rizolex-T 50%, with a reduction of 34% and 27%, respectively compared with those grown in soil infected with FOP. A similar pattern was shown in plants treated with disinfectant agents at different concentrations, with higher reduction in CAT activity by 33%, 33%, and 22% recorded in plants treated with chloroxylenol 1.2%, phenol 1.2% and phenic 1.2%, respectively. In addition, POD activity was reduced by 28%, 27%, and 24% in plants treated with phenic 1.2%, chloroxylenol 1.2% and phenol 1.2%, respectively.

Table (8). Effect of disinfectant agents formulated as DP 20% on activities of catalase and peroxidase enzymes of bean seedlings infected by FOP

Treatments	Catalase	Peroxidase
	(Unit/min/mg %)	(Unit/min/mg %)
Control infected soil	0.75±0.03^{a}	0.67±0.03^{a}
Control sterilized soil	0.40±0.01^{e}	0.39±0.04^{e}
Phenol at 0.4%	0.63±0.05^{bc}	0.57±0.03^{bc}
Phenol at 0.8%	0.55±0.06^{b-d}	0.53±0.03^{b-d}
Phenol at 1.2%	0.50±0.03^{d}	0.51±0.01^{cd}
Chloroxylenol at 0.4%	0.58±0.03^{b-d}	0.58±0.01^{b}
Chloroxylenol at 0.8%	0.53±0.01^{cd}	0.50±0.03^{cd}
Chloroxylenol at 1.2%	0.50±0.02^{d}	0.49±0.03^{d}
Phenic at 0.4%	0.65±0.01^{ab}	0.52±0.01^{b-d}
Phenic at 0.8%	0.61±0.02^{bc}	0.49±0.01^{d}
Phenic at 1.2%	0.58±0.05^{b-d}	0.48±0.01^{d}
Rizolex-T 50%	0.49±0.04^{de}	0.49±0.03^{d}
p-value	*	*

* Values are means ± standard error (n = 3). * and ** indicate differences at $p \leq 0.05$ and 0.01 probability level following Tukey's post hoc test.

4. Discussion:

The physicochemical properties of phenol were conducted to determine the optimal formulation type (Table 1) **(Hussien *et al,* 2022)**. To select an appropriate diluent, the pesticide formulator must possess knowledge regarding the specific characteristics of the diluent that align with the features of the active component **(Gnedy, 2016; Hamouda *et al.,* 2022)**. Furthermore, the physicochemical characteristics of the diluent utilized (Table 2) passed through a 74-micron test sieve **(CIPAC, MT59.1, 2002)**. Furthermore, the bulk density (%) pre- and post-compaction mustn't exceed 60% of the density pre-compaction.

Sorbents like talc, clay, or chalk are finely ground solid inert that are combined with an active substance to create dust. Since no mixing is needed, they are comparatively simple to use and the application equipment is lightweight and easy to use (such as hand bellows and bulb dusters). It can be observed that the particle size of these formulations is greater (about 25 to 35µ) than that of wettable powder formulations (roughly 5 to 10µ) since they are not

diluted with water prior to field application **(Hazra and Purkait, 2019)**.

Talc is suitable as a diluent for making dustable powder, based on data from Table (2). Following **(FAO/WHO MT., 2010)**, all tested dustable powders passed through a 74-micron test sieve with success, according to data shown in Table (3). Both the pre-compaction and post-compaction bulk densities were done following **(W.H.O., 1979)**, which stated that the powder's bulk density following compaction should not exceed 60% of its pre-compaction value.

Phaseolus vulgaris L. holds significant importance as a leguminous crop in Egypt, for both local consumption and exportation purposes **(Nemli *et al*., 2015)**. Damping-off and Fusarium wilt diseases caused by *Fusarium oxysporum* f. sp. *phaseoli*, are serious and persistent challenges for bean production. These diseases result in substantial crop losses, ranging from 50-100%, primarily due to premature wilting **(Schwartz *et al*., 2005)**. Management of fusarium wilt in susceptible cultivars poses significant challenges, with a few recommended management strategies **(Sidawi *et al*., 2010)**. Using repeated fungicides facilitates the development of fungicidal resistance. Hence, there is an increasing necessity to devise alternative approaches for controlling plant diseases instead of relying on pesticides.

Disinfectants are chemical compounds that can eliminate infectious microorganisms, excluding bacterial spores **(Block, 2001)**. Certain disinfectants that are frequently employed have been found to exhibit little efficacy against fungi. In addition, it noted that not all fungal species exhibit the same level of sensitivity to a particular substance. Furthermore, even different strains of the same fungal species might display varying degrees of resistance **(Jeffrey, 1995)**. Therefore, according to the British Standards Institution, disinfection is not the complete killing of all microorganisms, but rather the process of reducing them to a level that is acceptable for a certain purpose. This level is considered acceptable as it does not cause any risk to health or the quality of goods.

Results of the present study indicate that the application of commercial disinfectant agents (phenol, chloroxylenol, and phenic) formulated as 20% dustable powder at different concentrations (200, 400, 600, 800, and 1000 ppm) was effective in reducing the linear growth of *Fusarium oxysporum* f. sp. *phaseoli in vitro*. Chloroxylenol formulated was the most efficient tested compound in this regard. It achieved the lowest value in EC_{50} which was estimated at 351.15ppm, while EC_{50} values for both phenol and phenic formulated were 628.14 and 832.39ppm, respectively. **Gupta *et al.* (2002)** proved that phenol 5% was effective against Candida species. While, **Abed and Hussein (2016)** found that Dettol has greater efficacy than formalin against fungi, but is less effective against bacteria. Chloroxylenol (Dettol) at 10% has significant antifungal activity against *Aspergillus flavus.* **Mohammed and Al-Jibouri (2015)** reported that the optimal concentration of Dettol for all identified fungus was found to be 10%. Additionally, **Poger and Mark (2019)** found that the mode of action of chloroxylenol is similar to other phenolic and halo phenolic antimicrobial agents, particularly those disrupting cell membranes and inducing cell leakage. Furthermore, **Farzana et al. (2011)** showed that an elevation in the concentration of the active ingredient, chloroxylenol, led to the coagulation of functioning proteins and nucleic acids within the cell, ultimately resulting in rapid cell death.

Conclusion:

Bean seeds treated with disinfectant agents formulated as DP 20% and Rizolex-T 50% suppressed fusarium wilt disease under greenhouse conditions. Rizolex-T 50% improved the chemical constituents of bean plants, but still is lower than the plants grown in sterilized soil. Disinfectant agents formulated as DP 20% improved plant growth and encouraged the formation of defensive substances that reduced biotic stress, in addition, accelerated plant growth. In conclusion, chloroxylenol or phenol formulated as DP 20% at a concentration of 1.2% controlled *Fusarium oxysporum* f. sp. *phaseoli* and improved the growth characteristics of common bean plants.

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التأثير التثبيطي للمطهرات التجارية والعوامل المعقمة المصنعة على شكل مسحوق قابل للغبار ضد الذبول الفيوزارمي في نباتات الفاصوليا

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الملخص العربى:

مرض الذبول الفيوزارمي الذي يسببه فطر *Fusarium oxysporum* f. sp. *phaseoli* (FOP) يسبب خسائر كبيرة في نباتات الفاصولياء من حيث الكمية والنوعية. عالمياً، هناك اتجاه لاختبار مواد آمنة وفعالة بأقل تركيز ضد الفطريات المسببة للأمراض النباتية لاستخدامها في المجال الزراعي كبديل للمبيدات. وبالتالي، تهدف هذه الدراسة إلى تقييم فعالية تركيبة المسحوق القابل للغبار بنسبة ٢٠٪ لبعض العوامل المطهرة التجارية (الفينول، الكلوروزيلينول، والفينيك) ضد FOP في الحقل والمعمل. تم قياس النشاط المضاد للفطريات في المعمل بتقنية الأغذية المسمومة بخمسة تراكيز هي ٢٠٠، ٤٠٠، ٦٠٠، ٨٠٠، و ١٠٠٠ ملجم/لتر. تم اختبار المواد المطهرة المحضرة على الجسم الحي عن طريق تغطية بذور الفاصولياء (صنف الجيزة ٦) بثلاثة تراكيز ٠,٤، ٠,٨، و١,٢% قبل الزراعة في التربة الموبوءة بـ FOP مقارنة مع50% Rizolex-T ، WP بالجرعة الموصى بها (٣جم/ كجم بذور). تم فحص الوزن الطازج والجاف، طول المجموع الخضري والجذري، المكونات الكيميائية، والنشاط الإنزيمي (الكاتلاز والبيروكسيديز) لشتلات الفاصوليا المصابة بالـ FOP بعد ٤٥ يوماً من الزراعة. أثبتت النتائج أن الكلوروزيلينول كان أكثر فعالية ضد FOP مقارنة بالمواد الأخرى التي تم اختبارها في المعمل، حيث كان EC50 351.15 ملغم/لتر، في حين كان الفينول والفينيك ٦٢٨,١٤ و٨٣٢,٣٩ ملغم/لتر، على التوالي. في الجسم الحي، أدت العوامل المطهرة التي تم تركيبها بنسبة ٢٠% و 50% Rizolex-T بتركيزات مختلفة إلى تقليل حدوث المرض بشكل ملحوظ، وزيادة طول المجموع الخضري والجذري، وكذلك زيادة الوزن الطازج والجاف. أيضاً أدى إلى زيادة معنوية في السكريات الكلية الذائبة وتركيز الكلوروفيل. بالإضافة إلى ذلك، فقد خفضت مستويات أنشطة البرولين والإنزيمات. تم تسجيل أعلى انخفاض في حدوث مرض الذبول الفيوزاريومي وزيادة في مؤشرات النمو وكذلك التركيب الكيميائي في شتلات نباتات الفاصولياء المعاملة بالكلوروزيلينول بنسبة ٠,٨ و١,٢% يليها الفينول بنسبة ١,٢%، في حين وجد أن الفينيك هو أقل المعاملات فعالية. ولذلك، يمكن استخدام الكلوروزيلينول ٢٠٪ تجاريًا للسيطرة على مرض ذبول الفيوزاريوم وزيادة جودة وكمية نباتات الفاصوليا لأنه واعد ضد مسببات الأمراض، وآمن، وأقل تكلفة من مبيدات الفطريات.

الكلمات الدالة: *fusarium oxysporum* f.sp *phaseoli* – الكلوروزيلينول – الفينيك – الفينول - تأثير مضاد للفطريات - نباتات الفاصوليا.