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ORIGINAL ARTICLE

# The role of storage on Mancozeb fungicide formulations and their antifungal activity against *Fusarium oxysporium* and *Rhizoctonia solani*

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Enzyme;
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**Abstract** The stability of fungicides Dicozeb 80% WP (Mancozeb), Sand flower 72% WP (Cymoxail + Mancozeb) and Triomax 66% WP (Cymoxail + Mancozeb + Copper oxychloride) were tested before and after storage at 54 ± 2 °C for 14 days and investigated their activity on radial growth of *F. oxysporum* and *R. solani in vitro* by Poisoned Food Technique. The results indicated that slightly increased of degradation rate of the active ingredient of Triomax followed by Sand flower and Dicozeb. The fungicidal activity of Mancozeb increased when mixed with Cymoxanil and Copper oxychloride by 97.4 and 73.4 times against *F. oxysporum* and *R. solani,* respectively. While Cymoxanil increased the activity of Mancozeb by 39.5 and 45.9 times against tested fungi *F. oxysporum* and *R. solani,* respectively. In addition, degradation of tested fungicides caused increase the mean activity of polyphenol oxidase enzyme and mean proline amino acid in mycelium of tested fungi. With another point of view, the present of Cymoxanil + Copper oxychloride in mixture of Triomax caused decrease in degradation of fungicidal activity of Mancozeb up on other tested fungicides.



## 1. Introduction

Plant diseases play a vital role in the decline of natural resources in agriculture. Their causal agents include biotic and abiotic factors, genetic disorders and living infections agents such as fungi, bacteria, viruses, viroid's, phytoplasmas, nematodes, parasitic plants, and protozoan's (Agrios 2005). Approximately 20,000 species of fungal plant pathogens have been report and these fungi (Cooper 2007, Ong 2011) cause

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about 85% of plant diseases. Many fungal genera including *Fusarium*, *Alternaria*, *Botrytis*, *Helminthosporium*, *Penicillium*, and *Rhizoctonia* have proved as a harmful pathogenic fungi and cause huge loss of crop yield in quality and quantity world-wide (Boyraz & Ozcan 2006).

Fusarium wilt of tomato (*Lycopersicon esculentum* Mill.) caused by *Fusarium oxysporum* [(Schlecht.) f. sp. *lycopersici* (Sacc.)] Snyder et Hansen., is one of the most prevalent, serious diseases of tomato (Reis et al. 2005; Sudhamoy et al., 2009) and the disease is considered as one of the main soil-borne systemic diseases (Schwarz and Grosch 2003). It causes significant losses in tomato production in both greenhouse and field-grown tomates (Nusret and Steven, 2004). Among these diseases, *Rhizoctonia* black scurf and stem canker caused by the fungus *Rhizoctonia solani* Kühn, considered as a severe problem in all potato producing zones of the country (Sneh et al., 1991; Ahmad et al., 1995; Khan et al., 1995).

Application of fungicides is the most convenient and predominant way for disease control. Their use has made it feasible to enhance crop yields and food production. The efficacy of fungicides influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells (Reinprecht 2010; Peerzada et al., 2020).

Dithiocarbamate pesticides form an important class of organic fungicide used over the world to control many diseases in a variety of crops. They are of low acute toxicity, but some of Ethylenebisdithiocarbamate ($EBDC_S$) manb, zinab, mancozeb, metiran and nabam can decompose to give another ethylenthiourea has been shown to possess carcinogenics, mutagenic, goitergenic and tetratogenic activates in animals, (Gronogrorac and schwack, 2009; Larsson et al., 1976).

Mancozeb was one of the compounds in three combinations, which proved best in present finding. Therefore, in these combinations the efficacy may be due to Mancozeb (Bhaliya and Jadeja, 2014).

Ethylenebisdithiocarbamate ($EBDC_S$) fungicide applied to crop to prevent disease such as early blight, late blight and anthracnose, with other diseases such as bacterial speak and bacterial spots controlled by the application of copper salts. Mixed sprays of $EBDC_S$ and copper found to give better results bacterial speak than copper alone. $EBDC_S$ applied as their manganese and zinc complex Maneb or Mancozeb on the crop. The solubility activity and stability of the dithiocarbamate complex is depend on metal ion (Singh and Tripathi 1982). Combing the Cymoxanil with other fungicides belonging to different mode of action has helped prevent the selection of strains pathogens resistant to this fungicide. Therefore, it is used in two or in three ways mixture with protestants systemic fungicide (Gisi, 1996).

The present study aimed to evaluate the effect of the storage at 54 ± 2 °C for 14 days on fungicides Dicozeb (Mancozeb), Sand flower (Cymoxail + Mancozeb) and Triomax (Cymoxail + Mancozeb + Copper oxychloride) stability as well as their efficacy against the mycelial growth of *R. solani* and *F. oxysporum* tested under *in vitro* condition.

## 2. Materials and methods

### 2.1. Chemical fungicides used

Three commercial fungicides used in this study were obtained from the Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt and tabulated in Table 1.

### 2.2. Chemicals

Sodium thiosulfate, potassuim iodid, sulfuric acid, nitric acid methanolic potassuim, leadacetate, silver nitrate and all organic solvents, methanol and acetonitrile HPLC grade, Deionized water, Mancozeb 80% WP $(C_4H_6\ Mn\ N_2\ S_4)_x$ $(Zn)_y$ under trade name Dicozib, Cymoxanil $C_7M_{10}N_4O_3$ (4%) + Mancozeb (12%) + Copper oxychloride (50%) equal metallic copper 29% used under trade name Triomax 66% WP and Mancozeb 64% + Cymoxanil 8% used under trade name Sand flower 72% WP.

### 2.3. Apparatus

An equipment HPLC (Agilent 1200 sires was used DAD detector and C-18 column. The used of a fungicide formulation evaluated as the following, Cymoxanil determined at wave length detection 240 nm with mobile phase methanol: water (95: 5 v/v) and flow rate 0.5 ml /min with retention time 4.25 min. However, ethylenethiourea determined at wavelength detection 254 nm with mobil phase acetonitrile: Methanol (70: 30 v/v) with flow rate 1.3 ml / min and the retention time was 1.977 min as illustrated by Gamon et al., (1998) and CIPAC (1995). ICP-MS apparatus obtained from thermo fisher company USA to determine of heavy metals lead, cadmium and arsenic as the following forward power 1550w, nebulizer gas 0.8 L/min, injector 2 mm L.D quartz, interface Ni sampler and skimmer, Q cell He gas flow 4.8 ml / min Q cell KED voltage. 2 V and 75 AS dwell time 300 ms.

**Table 1** Fungicides used.

| Common name | Trade Name | Chemical name | Formulation | Manufacturer |
|---|---|---|---|---|
| Mancozeb | Dicozeb | Manganese ethylenebis ((dithiocarbamate) polymeric) complex with zinc salt. | 80% WP | Agrochem |
| a) Cymoxanil<br>b) Moncozeb | Sand flower | a)1-(2-cyano-2-metho xymino acetyl)-3-ethylurea.<br>b) Manganese ethylenebis ((dithiocarbamat) polymeric) complex with zinc salt. | 72% WP | Xi'an MTI Co., Ltd |
| a) Cymoxanil<br>b) Mancozeb<br>c) Copper oxychloride | Triomax | a)1-(2-cyano-2-metho xymino acetyl)-3-ethylurea.<br>b) Manganese sthylenebis ((dithiocarbamate) polymeric) complex with zinc salt.<br>c) Dicopper chloride trihydroxide. | 66% WP | Agria S. A. |

### 2.4. Experiment work

#### 2.4.1. Storage stability test at 54 ± 2 °C

The tested fungicides formulation were stored for 14 days at 54 ± 2 °C by the method of (CIPAC 2000). Samples taken before and after storage to determine the active ingredients and relevant impurities for tested fungicide.

#### 2.4.2. Formulation

Standard preparation of tested fungicides and impurities individual standard solutions were prepared in methanol for each fungicide.

#### 2.4.3. Sample Preparation

All fungicides sample before and after storage extracted with methanol and filtrated to analyze by HPLC to determine Cymoxanil and ethylenethiourea percentage.

Determination of metallic copper and heavy metals: The metallic copper percentage was determined before and after storage according to (CIPAC 1967). Cuprrasing was formed by digestion with nitric acid afford with potassium iodide, cupreous iodide, the latter is titrated with sodium thiosulphate. While lead, cadmium and arsenic determined by ICP-Mass apparatus according to (CIPAC 1970).

Determination of Mancozeb by iodometric titration and the active ingredients (a.i) was determined before and after storage by decomposition with acid. Hydrogen sulphide is removed by silver nitrate (in case Mancozeb modified Copper) or by lead acetate (in case Mancozeb alone). Carbondisulphide ($CS_2$) evolved is absorbed methalonic potassium hydroxide and determined iodometric titration according to modified method of (CIPAC 1973).

### 2.5. Fungal strains used

Pure cultures of *Rhizoctonia solani* and *Fusarium oxysporium* supplied from the department of Fungicides, Bactericides and Nematicides, (Central Agricultural Pesticides Laboratory), Agricultural Research Center (A. R. C.).

### 2.6. Efficacy tested fungicides on pathogenic fungi

Three different fungicides Dicozeb (Mancozeb), Sand flower (Cymoxail + Mancozeb) and Triomax (Cymoxail + Mancozeb + Copper oxychloride) were tested at concentrations of 1000, 500, 400, 300, 200 and 100 ppm before and after storage and evaluated on radial growth of test fungus by Poisoned Food Technique (Mohanty et al., 2012; Moreno-Gavira et al., 2020). Stock solutions of the fungicides were prepared in sterile distilled water and added separately to get the required concentrations thin mixed with 50 ml of sterilized PDA medium and transferred equally into three Petri dishes. The media allowed solidifying. The plates prepared without fungicides served as control. These plates inoculated with 5-mm disc of 7 days old culture of the test fungi. All dishes incubated at 27-±2 °C for 7 days and radial growth of colony measured when the mycelia of control had filled the petri dishes. Each treatment performed in triplicate

The fungal growth inhibition, which calculated due to treatment against control using the following formula: (Satya et al., 2014).

Inhibition of growth (%) = R-r/R *100.

R is the radial growth of fungal mycelia in the control plate.

r is the radial growth of fungal mycelia in the treated plate.

### 2.7. Statistical analysis

The concentrations inhibition regression lines drawn according to the method of Finney (1971).

### 2.8. Effect of some fungicides ($EC_{50}$) before and after storage at 54 ± 2 °C for 14 days on amino acid proline and polyphenol oxidase enzyme produced by Rhizoctonia solani and Fusarium oxysporium

Fungicides (Mancozeb, Cymoxail + Mancozeb and Cymoxail + Mancozeb + Copper oxychloride) at $EC_{50}$ of each fungus was added to 50 ml sterilized (PD) medium inoculated with 3 discs (5 mm) of any fungus *Rhizoctonia solani* or *Fusarium oxysporium*. Three flasks used as replicates. All flasks were incubated at 27 ± 2 °C. When the mycelial growth covered the surface, in the untreated flask. The mycelial matrix excluded by filtration and mycelial mates were homogenized and carried out.

### 2.9. Determination of polyphenol oxidase activity

Activity of polyphenol oxidase was determined according to the colorimetric procedure adopted by Esterbaner et al., (1977).

### 2.10. Quantitative assay of the amino acid proline

Amino acid proline determined according to the colorimetric procedure adopted by Bates et al., (1973).

## 3. Results and discussions

Data in Table 2 shown the degradation rate of the active ingredient of Mancozeb, when Dicozeb was stored at 54 °C for 14 days. The initial value at initial time (zero time) was 79.42 % before storage and decreased to 76.35 % (i.e 3.86 % loss) after 14 days of storage. In addition, in the same Table 1 result showed that the rate of formation ethylenethiourea (Etu) was 0.07 % at one day while, it increased to 0.122% (i.e 74.3% increase) after 14 days from storage and became conformity with (FAO 2010). (Maximum value of Etu 0.5 %). These results are in agreement with those results obtained by (IARC 2001). Ethylenethiourea content in Ethylenebisdithiocarbamate ($EBDC_s$) fungicides formation depends on the pesticides storage conditions and it increased by increasing temperature, a moisture and length of storage period (Lentza-Rizosh, 1990; Camoni et al., 1998; Loper-Fernande et al., 2017).

The data in Table 3 shown the degradation rate of two active ingredients Mancozeb and Cymoxanil when Sand flower 72 % WP formulation was stored at 54 °C for 14 days. The initial value at initial time (zero time) was 63.95 % and 7.98 %

**Table 2** Effect of storage thermal stability of Mancozeb and its impurities Ethylenethiourea (Etu) in Dicozeb 80 % WP at 54 ± 2 °C.

| Storage periods (days) | Mancozeb 80% (WP) | Ethylenethiourea (Etu %) impurities in Mancozeb 80 % (WP) |
|---|---|---|
| 0 | 79.42 | 0.07 |
| 14 | 76.35 | 0.122 |

0 = Initial time before storage.

and decreased to 63.54 % and 7.81 % (i.e 0.64 % loss for Mancozeb and 2.13 % loss for Cymoxanil), respectively after 14 days of storage at 54 °C. According to FAO specifications FAO (2010) and FAO (2006). The tested fungicide Sand flower 72% WP becomes conformity with FAO.

In addition, Results in Table 3 showed that the rate of formation ethylenethiourea was 0.111% at one day and decreased to 0.073 % (i.e. 34.2 % loss) after 14 days of storage at 54 °C ± 2. It found that Etu (impurity) in Sand flower 72 % were bellowing matching the maximum level (0.5 %) defined by (FAO, 2010).

The data in Table 4 shown the degradation rate of three active ingredients (a.i) Mancozeb, metallic copper and Cymoxanil when Triomax 66% WP formulation was stored at 54 °C for 14 days. The initial value at zero time was 11.83, 28.82 and 3.95 % and decreased to 11.63, 28.23 and 3.89 % (i.e 1.69 % loss for Mancozeb, 2.05 % loss for metallic copper and 1.52 % loss for Cymoxanil), respectively after 14 days of storage. According to FAO specification FAO (1989), FAO (2006) and FAO 2010, respectively. The tested fungicide Triomax 66 % WP become conformity with FAO.

Data in Table 4 illustrated the effect of thermal storage at 54 °C on relevant impurities of Ethylenethiourea (Etu) % for Mancozeb, result showed that the rate of formation Etu was 0.22% at zero time and reached 0.227 % after 14 days from storage (i.e 3.18 % increase) and become conformity FAO (2010) (FAO max 0.5 %).

In addition, in Table 4 relevant impurities of lead, cadmium and arsenic mg/kg for copper oxychloride when Triomax 66 % WP was stored for 14 days at 54 °C. Results indicated that there is slightly decrease in heavy metals lead and arsenic content with extending times of storage. The initial value of lead, cadmium and arsenic for one day were 13.77, UND and 3.02 mg/kg and reached to 13.59, UND and 2.96 mg/kg detected of lead, cadmium and arsenic were 0.5 × X mg/kg, 0.1 × X mg/kg and 0.1 × X mg/kg, respectively. Where X is the copper content g/kg, the lead and arsenic as relevant. Impurities became conformity with FAO specification (1989) before and after storage at 54 °C It is similar that Mohamed et al., (2016) found that the amount of relevant impurities cadmium and arsenic of copper fungicide formulation wettable powder slightly affected by storage temperature and became conformity with FAO specification.

**Table 3** Effect of thermal storage at 54 ± 2 °C on Mancozeb, Cymoxanil and their impurities in Sand flower 72 % WP.

| Storage periods (days) | Mancozeb 64% | | Cymoxanil (a.i) % |
|---|---|---|---|
| | Mancozeb (a.i) % | Ethylenethiourea impurities % | |
| 0 | 63.95 | 0.111 | 7.98 |
| 14 | 63.54 | 0.073 | 7.81 |

0 = Initial time before storage.
% Etu = Ethylenethiourea in content Mancozeb × 100.

Obtained results were agreement with Suzanne Losage (1980) who reported that the formation of Ethylenethiourea by thermal degradation of ($EBDC_s$) in aqueous media greatly reduced the addition of copper salt. In addition, to evaluate of $CS_2$ and the decomposition of Mancozeb decreased when Mancozeb treated with nitrogen group, ester group or ketone group within the molecular structure. For example; 2,6 dimethylmorpholine in tridemorph; N, N-dialkyl aniline in benalaxyl; metalaxyl and oxadixyl or a cyano group, an amino group and an amide group in Cymoxanil which reduced the generation of (Etu) on long storage of the product and reduced the solublized Ethylenethiourea (Etu) during the manufacture or storage of Mancozeb. These process maintained or increased the fungicidal activity of Mancozeb and is more persistent in the field patents Hiroko-kobayashi et al. (1992); Hala et al., 2016; Simonethi et al., 2020.

### *3.1. Effect of tested fungicides before and after storage at 54 ± 2 °C for 14 days on the mycelial growth of F. Oxysporium in vitro*

Data in Table 5 indicated that generally Triomax (Mancozeb + Cymoxanil + Copper oxychloride) was the most effective fungicide against *F. oxysporium*. It recorded the lowest $EC_{50}$ value 4.921 ppm, followed by Sand flower (Mancozeb + Cymoxanil) with $EC_{50}$ value 116.04 whereas the highest $EC_{50}$ value found with Dicozeb (Mancozeb). It noticed from above results, the fungicidal activity of Mancozeb increased when it mixed with Cymoxanil and Copper oxychloride by 97.4 times while increased to 39.5 time when it mixed with Cymoxanil only. Vadhera et al., (1997) and Dar et al., (2013) Mancozeb and Zineb proved that they are the inhibitor of mycelial growth at all concentrations from 1000 to 2500 ppm while kapadiya et al., (2013) reported that (Cymoxanil + Mancozeb), (Carbendazim + Mancozeb) were most effective against *F. solani* and *F. oxysporium*. On the other hand, the effectiveness of tested fungicides deteriorated as result to storage at 54 ± 2 °C for 14 days with $EC_{50}$ values increased from 4.921 to 116.04, 191.66 to 169.93 and 150.8 to 814.99 in case of Triomax, Sand flower and Dicozeb, respectively. From going results, it concluded that copper oxychloride increased the effectiveness of Mancozeb and decreased the degradation of Mancozeb under storage conditions.

### *3.2. Effect of tested fungicides before and after storage at 54 ± 2 °C for 14 days on the mycelial growth of R. Solani in vitro*

Data in Table 6 indicated that generally Triomax (Mancozeb + Cymoxanil + Copper oxychloride) was the most effective fungicide against *Rhizoctonia solani* recorded the lowest $EC_{50}$ value 39.167 ppm, followed by Sand flower (Mancozeb + Cymoxanil) $EC_{50}$ value 79.527 whereas the highest $EC_{50}$

**Table 4** Effect of thermal storage at 54 ± 2 °C on Mancozeb, metallic copper, Cymoxanil and their impurities in Triomax 66% WP formulation.

| Storage periods (days) | Mancozeb 12 % | | Metallic copper 29 % | | | | Cymoxanil a.c 4 % |
|---|---|---|---|---|---|---|---|
| | Mancozeb a.c | Ethylene- thiourea % | Copper a.c % | Lead mg/kg | cadmium | Arsenic mg/kg | |
| 0 | 11.83 | 0.22 | 28.82 | 13.77 | UND | 3.02 | 3.95 |
| 14 | 11.63 | 0.227 | 28.23 | 13.59 | UND | 2.96 | 3.89 |

0 = initial time before storage. UND undetectable.

**Table 5** Effect of tested fungicides before and after storage on *F. oxysporium in vitro*.

| Concentrations (ppm) | Fungicides | | | | | |
|---|---|---|---|---|---|---|
| | Mycelial growth inhibition percentages | | | | | |
| | Dicozeb | | Sand flower | | Triomax | |
| | BS | AS | BS | AS | BS | AS |
| 1000 | 100 | 58 | – | 80 | – | – |
| 500 | 75 | 42 | 100 | 78 | – | **89.5** |
| 400 | 65 | 28 | 78 | 76 | 100 | **89.4** |
| 300 | 53 | 22 | 70 | 62 | 89.2 | **89.0** |
| 200 | 49 | 18 | 59 | 51 | 88.9 | **75** |
| 100 | 41 | 13 | 53 | 52 | 88.3 | **72** |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| $EC_{50}$ | 191.66 | 814.99 | 116.04 | 150.88 | 4.921 | **169.93** |
| $EC_{90}$ | 1018.1 | 6393.5 | 598.22 | 1713.8 | 33.865 | **476.75** |
| Slope | 1.767 ± 0.189 | 1.433 ± 0.19 | 1.799 ± 0.249 | 1.214 ± 0.177 | 0.833 ± 0.374 | **1.116 ± 0.265** |

(-): disappear BS: before storage AS: after storage.
(Dicozeb: active ingredient (mancozeb).
Sand flower: active ingredient (mancozeb + cymoxanil).
Triomax : active ingredient (mancozeb + cymoxanil + copper oxychloride).

**Table 6** Effect of tested fungicides before and after storage on *R. solani in vitro*.

| **Concentrations (ppm)** | **Fungicides** | | | | | |
|---|---|---|---|---|---|---|
| | **Mycelial growth inhibition percentages** | | | | | |
| | Dicozeb | | Sand flower | | **Triomax** | |
| | BS | AS | BS | AS | BS | **AS** |
| 1000 | 85 | 54 | – | 82 | – | **–** |
| 500 | 68 | 47 | 100 | 76 | – | **86** |
| 400 | 60 | 40 | 73 | 68 | 100 | **78.5** |
| 300 | 55 | 32 | 68 | 56 | 80 | **72** |
| 200 | 47 | 27 | 60 | 47 | 73 | **65** |
| 100 | 27 | 15 | 53 | 38 | 67 | **58.3** |
| Control | 0 | 0 | 0 | 0 | 0 | **0** |
| $EC_{50}$ | 147.064 | 745.011 | 79.527 | 603.52 | 39.167 | **58.138** |
| $EC_{90}$ | 285,077 | 39,373 | 126.953 | 2195.4 | 1150.2 | **236.55** |
| Slope | 0.995 ± 0.173 | 0.744 ± 0.17 | 1.456 ± 0.256 | 1.035 ± 0.175 | 0.873 ± 0.25 | **2.103 ± 0.415** |

(-): disappear BS: before storage AS: after storage.
Dicozeb: active ingredient (mancozeb).
Sand flower: active ingredient (mancozeb + cymoxanil).
Triomax: active ingredient (mancozeb + cymoxanil + copper oxychloride).

value was found with Dicozeb (Mancozeb). From the above results, it noticed that the fungicidal activity of Mancozeb increased when mixed with Cymoxanil and Copper oxychloride by 73.4 times while this increase was 45.9 times when mixed with Cymoxanil only. On the other hand the effectiveness of tested fungicides were deteriorated as a result to storage at 54 ± 2 °C for 14 days the $EC_{50}$ values increased from 39.167 to 79.527, 147.064 to 58.138 and 603.52 to 745.011 in the case of Triomax, Sand flower and Dicozeb, respectively. From going results, it concluded that Copper oxychloride increased the effectiveness of Mancozeb and decreased its degradation under storage conditions.

Effect of tested fungicides ($EC_{50}$) before and after storage at 54 ± 2 °C for 14 days on amino acid proline and polyphenol oxidase enzyme produced by *R. solani* and *F. oxysporium*.

Data in Table 7 showed that before storage a slight increase found in amino acid proline in mycelium of *F. oxysporium* treated with Dicozeb. In contrast, a slight decrease in the same amino acid found in mycelium of *F. oxysporium* in the case of Sand flower and Triomax. On the other hand, the mean of proline amino acid increased with all tested fungicide after storage under 54 ± 2 °C for 14 days from 0.38340, 0.34085 and 0.28968 before storage to 0.72550, 0.74423 and 0.99045 after storage in the case of Dicozeb, Sand flower and Triomax, respectively. The point of view, all tested fungicides slightly increased the activity of polyphenol oxidase in *F. oxysporium* up on control. The storage of these fungicides under 54 ± 2 °C for 14 days increased the activity of polyphenol oxidase from 0.033, 0.029 and 0.025 before storage to 0.076, 0.071 and 0.070 after storage in the case of Dicozeb, Sand flower and Triomax, respectively. Regarding the above results, it concluded that storage decreased the activity of tested fungicides this indication may be due to increase of the mean proline and poly phenol oxides activity in mycelium of fungi. With another point of view, the present of (Cymoxanil + Copper oxychloride) in mixture of Triomax caused a decrease in degradation of its fungicidal activity on other tested fungicides.

Data in Table 8 indicated that means of amino acid proline increased slightly in mycelium of *R. solani* that treated with Triomax compared with control. On the other hand, the mean of proline increased with all tested fungicides that stored under 54 ± 2 °C for 14 days from 0.42542, 0.44360 and 0.58968 before storage to 0.74550, 0.80022 and 0.82045 after storage in the case of Dicozeb, Sand flower and Triomax, respectively. In addition, the mean activity of polyphenol oxides increased in mycelium as resulting of treatment with all tested fungicides up on control. The highest increase found with Dicozeb followed by Triomax and Sand flower. In contrast, the activity of polyphenol oxides increased in mycelium when treated with the same fungicide that stored under 54 ± 2 °C for 14 days from 0.030, 0.031 and 0.037 to 0.066, 0.070 and 0.085 in case of Dicozeb, Sand flower and Triomax, respectively. Regarding the above results, it concluded that degradation of tested fungicides increased the role of these fungicides in increasing the mean activity of polyphenol oxides and mean amino acid proline in mycelium of tested fungi.

Generally, the commonly available fungicides are effective against plant pathogens. The obtained results indicated that Mancozeb and combination of fungicides (Mancozeb + Cymoxanil), (Mancozeb + Cymoxanil + Copper oxychloride) had high efficiency to control the pathogen. The alternate application of these chemicals reduced the risk of development of resistant in pathogen similar result recorded by El Habbaa et al. (2002).

**Table 7** Effect of tested fungicides ($EC_{50}$) before and after storage at 54 ± 2 °C for 14 days on amino acid proline and polyphenol oxidase enzyme (PPO) produced by *F. oxysporium*.

| **Fungicides** | | ***Fusarium oxysporium*** | |
|---|---|---|---|
| | | Mean Activity of polyphenol oxidase | **Mean of amino acid proline µg/0.1 g mycelium** |
| Dicozeb | (BS) | 0.033 | **0.38340** |
| | (AS) | 0.076 | **0.72550** |
| Sand | (BS) | 0.029 | **0.34085** |
| flower | (AS) | 0.071 | **0.74423** |
| Triomax | (BS) | 0.025 | **0.28968** |
| | (AS) | 0.070 | **0.99045** |
| | Control | 0.020 | **0.34293** |

BS: before storage. AS: after storage.

**Table 8** Effect of tested fungicides ($EC_{50}$) before and after storage 54 ± 2 °C for 14 days on amino acid proline and polyphenol oxidase enzyme (PPO) produced by *R. solani*.

| Fungicides | | *Rhizoctonia solani* | |
|---|---|---|---|
| | | Mean Activity of polyphenol oxidase | Mean amino acid proline µg/0.1 g mycelium |
| Dicozeb | (BS) | 0.030 | **0.42542** |
| | (AS) | 0.066 | **0.74550** |
| Sand | (BS) | 0.031 | **0.44360** |
| flower | (AS) | 0.070 | **0.80022** |
| Triomax | (BS) | 0.037 | **0.58968** |
| | (AS) | 0.085 | **0.82045** |
| | Control | 0.032 | **0.45253** |

BS: before storage. AS: after storage.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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