Biological Impact, Formulation and Field Performance of Some Benzimidazole Derivatives as Novel Plant Protective Agents

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Abstract: Fungicidal evaluation for new ten benzimidazole derivatives, belonging to three different functional groups attached to C-2 of benzimidazole ring system, was carried out in this study aginst four pathogenic fungi. The tested fungi are *Rhizoctonia solani, Fusarium oxysporium, Botrytis allii* and *Aspergillus niger.* Some of the investigated compounds possessed promising antifungal activities. Structural activity relationship was discussed in this work. The most reactive derivative was formulated in the form of 10% Emulsifibale Concentrate (EC). The new formulation was re-evaluated and its activity was compared with the standard fungicide. Field performance efficacy of the local formulation against some pathogens that cause bulb rotting of onion during storage was also investigated. Data obtained under laboratory conditions showed that, some new benzimidazoles possessed high biological impacts. Local formulation showed also higher activity than that of the standard fungicide under laboratory and field conditions.
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Key words: benzimidazole derivatives, soil borne fungi, air borne fungi, bulb rotting of onion and formulation.

1. Introduction

Fungal infections pose a major health hazards to the world's most important crops like rice, wheat, maize, potatoes, onion, sugarcane. These infections constitute a major reason for massive economic losses in most of crops throughout the world. Benzimidazole ring system is characterized by its broad spectrum of biological activity in pharmaceutical field **(Ramla *et al.*, 2006, Gowda *et al.*, 2009, Ingle *et al.*, 2011and Mukesh, 2010)**. In agricultural field, many benzimidazole derivatives were reported to be insecticides **(Joshi *et al.*, 1990 and Hansheng *et al.*, 1990)**, herbicides **(Heywang *et al.*, 1998**), acaricides **(Maki *et al.*, 1989**) and fungicides**.** As a result, global market involves many commercialized fungicides whose active ingredients are classified as benzimidazole family. Prompted by the previous facts and in continuation with our recently published works **(Madkour *et al.*, 2006 and Ibrahim 2008)**, the current study benzimidazole derivatives against four target fungi attacking many crops in Egypt and all over the world either in pre-harvest or post-harvest stages.

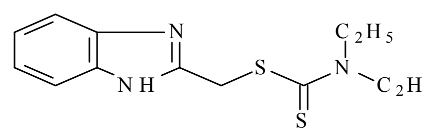
2. Materialsand Methods

1- The standard fungicide used.

Tecto 50% w.p (thiabendazole). Its active ingredient belongs to benzimidazole family and is recommended to fight some air borne fungi that attack onion during its storage stage **(Rajapakse *et al.*, 2004)**.

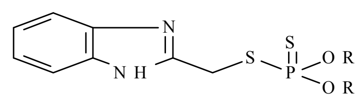
2- The new synthesized compounds.

Ten samples of different benzimidazole derivatives that have been recently published were prepared according to the reported method **(Madkour *et al.*, 2006 and Ibrahim, 2008)**. These derivatives belong to three different functional groups which are dithiocarbamate, dithiophosphate and aryloxy benzimidazole. The structural formulae of the prepared compounds are shown as follows:-

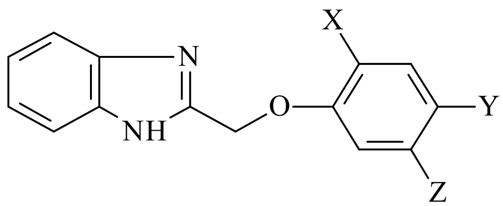


(1)

(2)



Compound No.	R
2a	CH_3
2b	CH_2CH_3
2c	$CH_2CH_2CH_3$



(3)

Compound No.	X	Y	Z
3a	Cl	Cl	H
3b	Cl	NO_2	H
3c	NO_2	Cl	H
3d	F	F	H
3e	NO_2	Br	H
3g	H	NO_2	CH_3

3- *In vitro* test.

Potato-dextrose agar (PDA) was used to evaluate the effect of the selected compounds under investigation on the mycelial linear growth of the four tested fungi. Fifty milliliters of the aforementioned medium were poured into 150 ml conical flasks and autoclaved at 121°C for 20 min. Three drops of 25% lactic acid were added to prevent bacterial contamination. A series of different concentrations for each compound was prepared (v/v) by dissolving appropriate amounts of this compound in 10 ml DMSO. Equal volumes of DMSO containing diluted compounds were added to sterile molten (40°C) PDA to get a series of concentrations of 50, 75, 100, 150, 250, 500 and 750 ppm for each compound in PDA **(Tremblay *et al.*, 2003)**.

A zero (0) concentration treatment was prepared for each fungus, which contains equivalent volume of solvent only, was used as a control. Compounds-amended PDA were dispensed aseptically into 9 cm diameter petridishes. Plugs of mycelium (4 mm diameter) were cut from the margins of actively growing cultures of the *Rhizoctonia solani, Fusarium oxysporium, Botrytis allii* and *Aspergillus niger* fungi and placed in the center of compound-amended and unamended PDA plates with 4 replicate plates for each fungus. All plates were incubated at 25 ± 1°C. Colony diameter (in millimeters) was measured after complete growth of control plugs for each fungus. Similarly, fungicidal activity of both standard and local formulations was carried out where water was used as a diluent. The percentage of growth inhibition was calculated for each compound. The estimated effective concentration (EC_{99}) that inhibits 99% of fungal radial growth, toxicity index (T.I.) and slopes of toxicity lines for each compound under investigation were determined and tabulated in tables (1- 4,6,7).

4-Field performance evaluation.

A series of concentrations from 10 ppm to 250 ppm were prepared. Fresh samples of flawless onion bulbs were selected and dipped separately in different fungicidal concentrations for 5 minutes before inoculated with both two fungi for 24 ± 2 hrs after treatment. This inoculation method simulates infections and has been recommended for determination the effectiveness of fungicides **(Srinivasan *et al.*, 2006)**. Bulbs were inoculated by immersing a stainless steel rod with a probe tip 1mm wide and 2 mm in length into the spore suspension and wounding each bulb. The temperature of the blub at the time of inoculation and subsequent storage until treatment was 20°C ± 1°C. One replicate consists of 40 bulbs was stored in paper sacs under room conditions. The observations were recorded after 3 months of inoculation by evaluating the percentages of infection and tabulated in tables (8 - 9) and figures (3-4).

3. Results and Discussion

I- Preliminary screening the fungicidal activity of the prepared compounds against the target fungi.

The synthesized benzimidazole derivatives were tested against *Rhizoctonia solani* as an example of a soil borne fungi in a series of concentrations 50, 75, 100, 150, 250, 500 and 750 ppm. The percentages of inhibition, effective concentrations (EC_{99}), toxicity indexes and slopes of the toxicity lines were calculated and represented in table (1). Data obtained revealed that no benzimidazole derivatives showed a complete inhibition for the fungal radial growth even at the highest concentration of 750 ppm which reflects the lower activity of the tested compounds toward this fungus. It was noted that all compounds gave over %90 inhibition for the fungal radial growth at the highest concentration. From the obtained data it is concluded that, compound **(3d)** is the most effective one, its EC_{99} being 527.3 ppm followed by compound **(3c)** its EC_{99} being of 542.4 ppm. Data obtained also indicate that the lowest effective compound is **(3a)** with EC_{99} value of 3784.8 ppm. Compounds **(2b)**, **(3e)** and **(2a)** showed moderate inhibitory effect against the tested fungus where, their EC_{99} values are 634.9, 729.4, 779.1 ppm and their toxicity indexes are 83.1, 72.3 and 67.7 respectively compared to the most potent one.

Table (1):- The fungicidal activity of new synthesized benzimidazole derivatives on *Rhizoctonia solani* as a soil borne fungus.

Conc. (ppm)	Inhibition %							EC_{99} (ppm)	T.I	Slope
Compd. No.	**50**	**75**	**100**	**150**	**250**	**500**	**750**			
1	1.8	6.8	14.5	32.8	62.9	91.6	97.7	937.3	56.3	3.48
2a	63.3	73.7	79.9	87.2	93.4	97.7	98.9	779.1	67.7	1.67
2b	44.8	60.3	70.5	82.4	92.3	98.2	99.4	634.9	83.1	2.23
2c	52.5	62.8	69.6	78.1	86.6	94.0	96.5	1648.5	31.9	1.49
3a	9.4	16.5	23.2	34.8	51.6	73.5	83.3	3784.8	13.9	1.94
3b	6.0	14.6	24.4	42.5	67.4	90.5	96.5	1131.2	46.6	2.87
3c	15.3	32.5	48.0	69.9	89.2	98.7	99.7	542.4	97.2	3.24
3d	7.8	21.9	37.5	62.8	87.3	98.7	99.8	527.3	100	3.66
3e	5.7	16.1	28.4	50.8	77.8	96.2	99.1	729.4	72.3	3.36
3f	14.1	27.1	38.9	57.3	77.9	94.1	97.9	970.8	54.3	2.64

For more details about the behavior of the screened compounds toward the soil borne fungi, fungicidal activity of the prepared compounds on *Fusarium oxysporum* fungus was investigated. Percentages of inhibition, EC_{99}, slopes of toxicity lines as well as toxicity indexes were calculated and tabulated in table (2). According to the obtained data it is clear that, benzimidazole derivatives **(3a)** is the most effective compound on this fungus. It gave a complete inhibition for the fungus radial growth at concentration of 250 ppm and it was found to have the lowest EC_{99} value. Compounds **(3b)** and **(3d)** displayed the same effect but at concentrations of 500 and 750 ppm respectively. It is noted that, at the highest concentration, all tested compounds displayed more than 90% inhibition for the fungal radial growth except compound **(1)** which gave 85.6% of inhibition with very high EC_{99} value, things that reflect the resistance of *F. oxysporum* fungus to the prepared benzimidazole derivative. Data in table (2) revealed that, fungicidal potency of the synthesized benzimidazole derivatives on *F. oxysporum* fungus can be arranged descendingly as follows: **(3a)**, **(3b)**, **(3d)**, **(3c)**, **(2c)**, **(2a)**, **(2b)**, **(3e)**, **(3f)**, and **(1)** where their toxicity indexes are 100, 72.5, 42.8, 24.5, 21.1, 16.6, 16.1, 13.2, 1.8, and 0.001 respectively.

The *Aspergillius niger* and *Botrytis allii* fungi are from the most destructive air borne fungi that cause a high loss in productions for many crops either in pre-harvest or post-harvest stages **(Kumar *et al*., 2015)**. In the current study the potency of the prepared compounds on these two fungi was screened. The *Aspergillius niger* fungus was treated with the same dosage of concentrations for seven days under laboratory conditions. The percentages of inhibition of the fungal radial growth, EC_{99} values, slopes of toxicity lines and toxicity indexes were calculated and presented in table (3). Data obtained show that the percentages of inhibition is directly proportional to the concentration of the tested compounds. Compound **(3c)** is the only derivative that gave a complete inhibition for the fungal radial growth at the highest concentration (750 ppm) and more than 97% for radial growth at 250 ppm. All screened compound possessed over 95% radial growth inhibitions at 500 ppm reflecting the sensitivity of *Aspergillius niger* fungus to these compounds. According to the estimated EC_{99} values and toxicity indexes, compound **(3c)** was found to be the most potent derivative on this fungus where, its EC_{99} value is 302.8 ppm followed by compounds **(2c)** and **(2b)** as their toxicity indexes are 77.6 and 75.1 respectively comparing with the most potent derivative **(3c)**.

n addition, *Botrytis allii* air borne fungus was treated with a series of concentrations for each synthesized benzimidazole ranging from 50 to 750 ppm and the obtained data presented in table (4). Quantitative and corrected data obtained clearly show that compound **(3c)** is the only derivative displaying a complete inhibition for the fungal radial growth at the concentration of 250 ppm. Moreover, at the lowest concentration (50 ppm) compound **(3c)** gave the highest inhibitory effect (80.15%), reflecting the high sensitivity of *Botrytis allii* fungus to benzimidazole derivative **(3c)**. With the exception of compounds **(2b)** and **(2c)**, all screened benzimidazoles possessed over 99% inhibitions for fungal radial growth at concentration of 500 ppm. Toxicity indexes and the estimated EC_{99} indicated that compound **(3c)** is the most effective one followed by compounds **(3a)** and **(3b)** as their EC_{99} values are 116.0, 147.8, 202.9 ppm and their toxicity indexes are 100.0, 78.5 and 57.2 respectively. On the other hand, compounds **(2b)** and **(2c)** was found to be the least reactive derivatives as their toxicity indexes are 6.5 and 8.9 respectively and their EC_{99} are over 1000 ppm.

Table (2):- The fungicidal activity of new synthesized benzimidazole derivatives on F. oxysporium as a soil borne fungus.

Conc. (ppm)	Inhibition %							EC_{99} (ppm)	T.I	Slope
Compd.No.	50	75	100	150	250	500	750			
1	63.1	66.9	69. 5	72.9	77.0	82.0	84.6	1.31×10^5	0.001	0.58
2a	6.8	18.7	32.2	55.6	81.6	97.3	99.4	652. 9	16.6	3.42
2b	1.7	7.7	17.4	40.4	73.6	96.5	99.4	673.7	16.1	3.94
2c	1.6	8.6	20.8	48. 7	82.9	98.9	99. 9	512.4	21.1	4.42
3a	64.9	91. 6	98.3	99.9	100.0	100.0	100	108.3	100.0	5.80
3b	76. 6	90.6	95.9	99.0	99.9	100.0	100	149.4	72.5	3.37
3c	67.4	78.8	85.3	91.9	96.7	99.3	99.7	441.8	24.5	1.98
3d	25.6	53.6	73.2	91.4	98.9	99.8	100	252.8	42.8	4.24
3e	45. 6	59.5	68.8	80.1	90.1	97.1	98.7	823.2	13.2	2.00
3f	60.0	66.6	71.0	76.7	82.9	89.5	92.3	5990.3	1.8	0.99

Table (3):-The fungicidal activity of new synthesized benzimidazole derivatives on *Aspergillus niger* as an air borne fungus.

Conc. (ppm)	Inhibition %							EC_{99} (ppm)	T.I	Slope
Compd. No.	50	75	100	150	250	500	750			
1	10.7	23.7	36.4	56.9	79.7	95.7	98.7	798.0	39.9	2.96
2a	0.5	3.2	10.4	33.2	72.9	97.8	99.7	578.0	53.3	4.71
2b	3.2	14.8	31.8	62.9	91.1	99. 7	99. 8	410.3	75.1	4.57
2c	2.6	13.4	30.5	63.1	91.9	99. 8	99.9	390.3	77.6	4.79
3a	4. 5	16.3	31.8	59.6	87.4	99.1	99.2	487.3	62.0	4.07
3b	15.3	33.5	49.9	72.4	91.1	99.1	99.8	485.8	62.2	3.39
3c	5.2	23.1	45.8	78.4	97.2	99.7	100.0	302.8	100.0	5.05
3d	10. 7	24.3	37.8	59.3	82.2	96.8	99.2	707.8	42.8	3.10
3e	11.0	26.8	42.6	66.2	88.2	98.7	99.7	537.4	56.3	3.44
3f	27.4	47.4	62.4	80.3	93.7	99.3	99.8	457.9	66.1	3.04

Table (4):- The fungicidal activity of the new synthesized benzimidazole derivatives on *Botrytis allii* air born fungus.

Conc. (ppm)	Inhibition %							EC_{99} (ppm)	T.I	Slope
Compd. No.	50	75	100	150	250	500	750			
1	60.9	79.6	88.8	96.2	99.3	99.9	100.0	226.4	51.2	3.12
2a	7.9	31.2	56.5	86.1	98.7	99.9	100.0	258.8	44.8	5.24
2b	3.6	9.1	15.8	29.6	52.2	80.4	90.7	1791.3	6.5	2.66
2c	4.9	12.4	21.1	37.8	62.4	87.7	95.1	1302.5	8.9	2.81
3a	68.2	87.8	95.1	98.1	99.2	100.0	100.0	147.8	78.5	3.94
3b	76.3	88.2	93.5	97.6	98.5	99.6	99.9	202.9	57.2	2.65
3c	80.2	94.1	98.1	99.2	100.0	100.0	100.0	116.0	100.0	4.05
3d	29.4	58.4	77.2	93.3	99.3	99.9	100.0	234.9	49.4	4.27
3e	69.5	83.4	70.2	96.0	98.7	99.9	99.9	249.0	46.5	2.61
3f	62.5	77.9	88.6	95.7	99.1	99.8	99.99	239.9	48.4	2.95

Generally, changing the functional groups attached to C-2 of benzimidazole ring system clearly changes its fungicidal potentiality toward the all tested fungi. Introducing an aryloxy functional group in position 2 of benzimidazole nucleus (structure 3) increases the fungicidal activity against the two air borne fungi to better rates than do the other functional groups. The optimum activity was found when chloro and nitro substituents were introduced in aryloxy moiety. In addition, substituent 4-chloro-2-nitro derivative **(3c)** was found to be the most effective one against *Aspergillus niger* and *Botrytis allii* air borne fungi. In case of soil borne fungi, benzimidazole derivatives **(3a)** and **(3d)** are more prevalent.

According to the previous results, compound **(3c)** is subjected to the process of formulation and re-evaluation in laboratory and field against *Aspergillus niger* and *Botrytis allii* fungi to achieve the target of this study.

II- Formulation of benzimidazole derivative (3c).

Pesticide formulation is a mixture of the biologically active substance, called active ingredient (A.I), with other inert substances. The biological activity of the pesticide is determined by its (A.I). Initially, formulation is the main technique used to apply the low mass (A.I) of pesticides over a large area in field. Pesticide products very rarely consist of pure technical material or (A.I). It is usually formulated with other materials and this is the product as sold, and may be further diluted in use. Formulation improves the properties of the (A.I) for handling, storage, application and may substantially influence its effectiveness and safety.

II.1- Components of the local formulation (10% EC).

Active ingredient (compound 3c)	10.00%
Anti foam (silicon)	0.20%
Wetting agent (tween 80)	1.30%
Surfactant (toximol 500) Solvent 1 (1-propanol)	2.47% 15.20%
Solvent 2 (dimethylformamide)	70.83%
Total	100.00%

II.2- Physico-chemical properties of the local formation.

Data in table (5) represents the physico-chemical properties of the local formulation in the three recommended types of water used in dilution (tap, soft and hard). The table implies that the values of the seven measured parameters depend on the type of water used. The formulation successfully passed the recommended tests according to the standard methods **(FAO and WHO joint meeting on pesticides specifications, 2006)**.

Table (5): Physico-chemical properties of the local formulation spray solution.

Test (unit)	**T. W.**	**S. W.**	**H. W.**
1- Conductivity (mmhos)	230.0	300.0	810.0
2- Salinity (%)	0.0	1.0	2.0
3- Surface tension (dyne/cm)	28.3	30.2	31.5
4- pH value	5.8	6.1	6.4
5- Viscosity (centipoises)	8.3	7.8	7.1
6- Foam (cm)	0.0	0.0	0.0
7- Emulsion stability	✓	✓	✓

II. 3- Fungicidal activity of the local formation under laboratory conditions.

Data shown in table (6) and figure (1) indicate the fungicidal activity of both local formulation (10% EC) and the standard fungicide tecto (50% SC) against the *Botrytis allii* fungus. It is clear that at concentration of 10 ppm local formulation displayed 17.3% of inhibition while the standard fungicide displayed 14.9%. Treatment of the current fungus with 100 ppm of two formulations demonstrated that the new formulation possessed over 99% inhibition for fungal radial growth while the standard fungicide gave about 97% inhibition. The advantage in activity of the local formulation is clearly realized by comparing its EC_{99} value with that of the standard fungicide. The calculated EC_{99} values were found to be 90.6 ppm and 121.2 ppm for local formulation and standard fungicide respectively.

Data in table (7) and figure (2) show the fungicidal activity of the local formulation (10% EC) and the reference fungicide (tecto 50% SC) on *Aspergillus niger* fungus under laboratory conditions. Both local and standard formulations possessed a lower activity toward *Aspergillus niger* fungus than they do toward *Botrytis allii* fungus. At concentrations of 10 ppm and 20 ppm, the two tested formulations displayed no activities in contrast with their activity toward *Botrytis allii* fungus. At concentration of 150 ppm the local formulation displayed 63.8% of inhibition and reference fungicide displayed 69.0%. The calculated EC_{99} values are 511.3 ppm for the local formulation and 502.1 ppm for the standard fungicide, reflecting the convergence in efficiency of the local formulation and the standard fungicide against *Aspergillus niger* fungus.

Table (6):-Fungicidal activity of the local formulation on Botrytis allii fungus as compared with the standard fungicide under laboratory conditions.

Conc. (ppm)	Local formulation (10% EC)			Standard formulation (50% SC)		
	Inhibition %	EC_{99} (ppm)	Slope	Inhibition %	EC_{99} (ppm)	Slope
10	17.3	90.6	3.42	14.9	121.2	3.11
20	53.4			45.8		
30	75.4			67.1		
40	86.7			79.7		
50	92.6			87.1		
75	97.8			95.4		
100	99.3			97.8		
150	99.8			99.3		

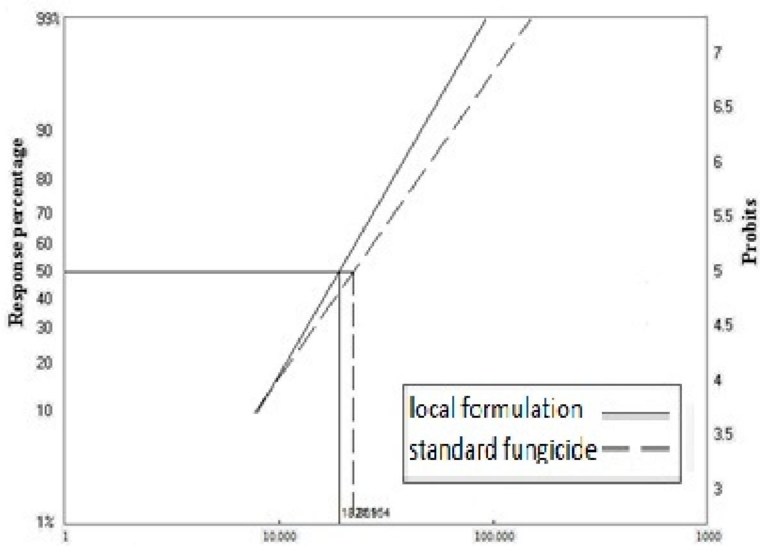


Figure (1):- Toxicity lines of both local formulation and the standard fungicide against Botrytis allii fungus in laboratory.

Table (7):- Fungicidal activity of the local formulation on Aspergillus niger fungus as compared with the standard fungicide under laboratory conditions.

Conc. (ppm)	Local formulation (10% EC)			Standard formulation (50% SC)		
	Inhibition %	EC_{99} (ppm)	Slope	Inhibition %	EC_{99} (ppm)	Slope
10	0.0	511.3	3.74	0.0	502.1	4.29
20	0.2			0.1		
30	1.2			0.6		
40	3.7			2. 5		
50	7.6			6.0		
75	22.0			21.3		
100	38.1			39.8		
150	63.8			69.0		
200	79.4			84.9		
250	88.2			92.6		
500	98.9			99.1		

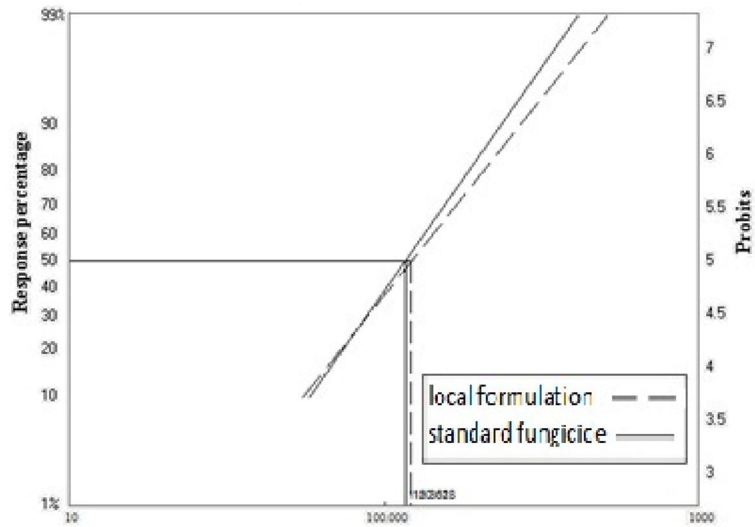


Figure (2):- Toxicity lines of both local formulation and the standard fungicide against Aspergillus niger fungus in laboratory.

II.4- Field evaluation of the local formulation.

In Egypt onion is an important crop with a constant demand. In order to manage its supply, long term storage is a prerequisite. High losses of onion during storage are mainly due to sprouting and contamination by several microorganisms. About 35-40 % onion is lost due to damage caused by storage diseases **(Kumar *et al.*, 2015)**. Among the predominant post-harvest diseases attacking onion are the black mould rot, caused by *Aspergillus niger* fungus, **(Srinivasn *et al.*, 2006)** and the neck rot disease, caused by *Botrytis allii* fungus **(Sayed *et al.*, 2014)**. Due to the higher activity of our local formulation against both *Aspergillus niger* and *Botrytis allii* fungi under laboratory conditions, its field efficiency was evaluated against the previous two pathogenic fungi.

Data presented in table (8) and illustrated in fig (3) represent the post-harvest efficiency evaluation of both local formulation (10% EC) and standard fungicide (tecto 50% SC) on *Botrytis allii* fungus during the onion storage process. Data obtained show that, with zero treatment, the percentage of infection is 100%, with zero percentage of inhibition, in case of the two applied formulations. As the concentrations of both formulations increase, the percentages of infection decrease.

Application of local formulation at rate of 100 ppm produces over 99% of inhibition for infected onion bulbs while the standard fungicide had almost the same effect at concentration of 200 ppm reflecting the advantage of the tested new formulation on the standard fungicide. The previous fact can also be proved by comparing the EC_{99} values of two formulations which are 99.5 and 158.3 ppm for local formulation and the standard fungicide respectively.

Table (8):- Post-harvest evaluation of both local formulation and the standard fungicide against *Botriytes allii* fungus during onion storage.

Conc. (ppm)	**Local formulation**		**Standard formulation**	
	% of infection	% of inhibition	% of infection	% of inhibition
Control	100.0	0.0	100.0	0.0
30	55.6	44.4	61.9	38.0
50	18.1	81.9	30.7	69.3
75	4.1	95.9	12.6	87.4
100	0.9	99.1	5.4	94.6
150	0.1	99.9	1.7	98.3
200	0.0	100.0	0.5	99.5
EC_{99} (ppm)	99.5		158.3	
Slope	4.74		3.64	

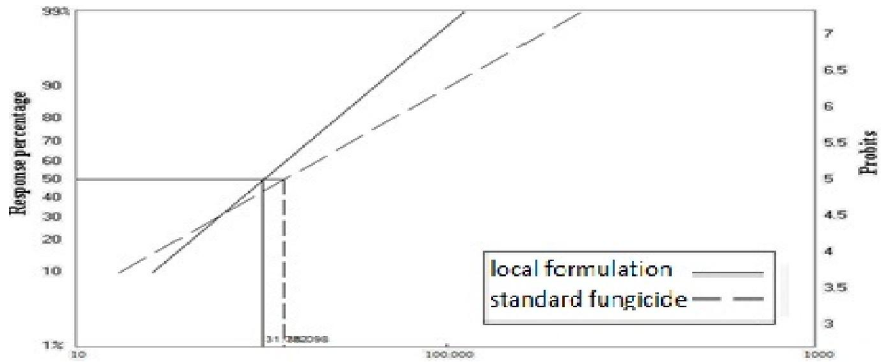


Figure (3):- Toxicity lines of local formulation and the standard fungicide against *Botrytis allii* in field.

Data presented in table (9) and fig. (4) demonstrate the post-harvest efficiency of both local formulation and the standard fungicide on *Aspergillus niger* fungus during the onion storage process. Data display that both formulations have nearly the same potentialities as they have close EC_{99} values. The calculated EC_{99} values are 294.1 and 282.7 ppm for local formulation and standard fungicide respectively.

Table (9):- Post-harvest evaluation of both local formulation and the standard fungicide on *Aspergillus niger* fungus during onion storage.

Conc. (ppm)	**Local formulation**		**Standard formulation**	
	% of infection	% of inhibition	% of infection	% of inhibition
Control	100.0	0.0	100.0	0.0
30	98.9	1.1	99.2	0.8
50	89.7	10.3	90.6	9.4
75	67.1	32.9	67.9	32.1
100	44.4	55.6	44.4	55.6
150	16.8	83.2	16.1	83.9
200	6.1	93. 9	5.5	94.5
250	2.3	97.7	1.9	98.1
EC_{99} (ppm)	294.1		282.7	
Slope	4.67		4.84	

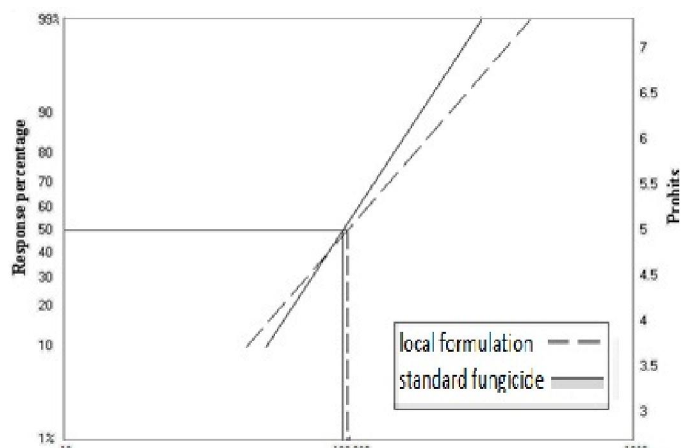


Figure (4):-Toxicity lines of local formulation and the standard fungicide against *Aspergillus niger* in field.

Conclusion

In summery, a promising newly published benzimidazole derivatives were prepared using widely available row martials. Introducing three different biologically active functional groups to C-2 of benzimidazole ring system and studying the fungicidal activity of these derivatives were carried out. The fungicidal activity of the prepared compounds was evaluated against four pathogenic fungi under laboratory and field conditions. Introducing the dithiophosphate functional group enhances the fungicidal activity especially against the air borne fungi. In some cases the new formulation displayed better activity than that of the standard fungicide. The current new formulation represents a good class of fungicides that are promising for further biological and toxicological studies.

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