Journal

J. Biol. Chem. Environ. Sci., 2010, Vol. 5(2):161-171
www.acepsag.org

BIOLOGICAL IMPACT OF SOME NEW FORMULATED PYRIDINETHIONE DERIVATIVES AS FUNGICIDES

Nasser A. Ibrahim*, Hanaa A. E.Attia and Saad E.S. Hamoda

Cent. Agric. Pest. Lab. (CAPL), Agricultural Research Center, Dokki, Giza, Egypt.

ABSTRACT

Fungicidal evaluation for twenty three new synthesized pyridinethione and thieno[2,3-b]pyridine derivatives was carried out on three tested fungi,i.e. *Sclerotium rolfesii, Rhizoctonia solani and Ramularia tulasnea.* Structure activity relationship was discussed in this work. Some of the investigated compounds possessed promising antifungal activities. The most effective derivative was formulated as 20% EC. The fugicidal activities of new formulation were evaluated in comparison with the commercial and recommended fungicides namely ridomil gold plus 42.5% WP(Copper oxychloride +Mefenoxam) (for *R.. tulasnea*) and moncut 25% WP (Flutolanil) (for *S. rolfesii* and*R.. solani*). Data obtained showed that, the new formulation showed higher activity than the commerical fungicide against.*(R.. tulasnea* and *S. rolfesii*)

Key words: pyridinthione, thieno [2,3-b]pyridine, plant pathogenic fungi and formulation,synthesis..

INTRODUCTION

Plant infection with fungi is cosmopolitan and affects many crops, the reason for continuous and great use of many toxic fungicides. As we are continuously use of fungicides, fungi have gradually acquired some resistance as reported with many fungi species (Arena *et al*., 1984, Sullia and Rose Maria, 1985 and Shim *et al*., 1998). Thus, the subject of searching for new fungicides is representing greatly importance and has the priority in the plant protection research field. Prompted by aforementioned facts, and in continuation with our laboratory research programs, we published

many investigations related to this search approach (Madkour *et al*., 2006, Youssef *et al*., 2008, Mohamed *et al.*, 2009 and Ibrahim 2008).

The present work is a complement of our recently reported one (Youssef *et al*., 2008) which studied the synthesis of some new pyridinethione and thieno[2,3-b]pyridine of expected bio-responses. The present study aimed to clarify the fungicidal activities of the new synthesized compounds against *Sclerotium rolfesii, Rhizoctonia solani* and *Ramularia tulasnea* fungi . In addition the new prepared formulation of the most potent compound was also evaluated from physical properties and fungicidal activity against the same fungi.

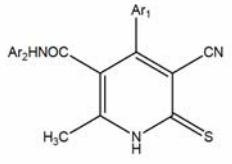
MATERIALS AND METHODS

1- The standard fungicides used.

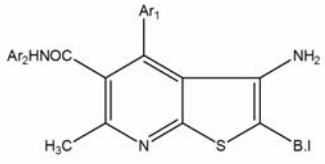
- Moncut 25% w.p (flutolanil*)* was used as a reference fungicide for the.*Sclerotium rolfesii* and Rhizoctonia *solani*.It was obtain from Nihon-Nohyaku-Japan(Manufacture).
- Ridomil gold plus 42.5% w.p (Copper oxychloride + Mefenoxam) was used as a reference fungicide for the *Ramularia tulasnea* It was obtain fromSyngenta .Agro-Switzerland. (Manufacture).

2- The new synthesized compounds

New twenty three 3-cyanopyridinethione and thieno [2,3-b]pyridine derivatives were prepared according to the method reported by (Youssef *et al*., 2008). The structural formulae of the prepared compounds are shown as follows:-



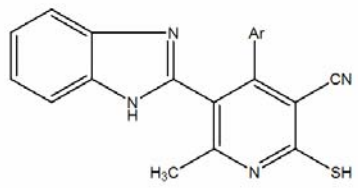
Structure (A)



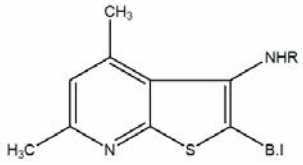
Structure (B)

Compd. No	Ar_1
A_1	4-F-C_6H_4
A_2	4-Cl-C_6H_4
A_3	2-furyl
A_4	4-Br-C_6H_4
A_5	3,4-$(MeO)_2$-C_6H_3
A_6	4-NO_2-C_6H_4

Compd. No	Ar_1
B_1	4-Cl-C_6H_4
B_2	4-F-C_6H_4



Structure (C)

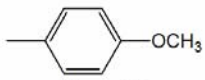


Structure (D)

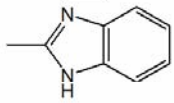
Compd. No	Ar
C_1	3,4-$(MeO)_2$-C_6H_3
C_2	4-MeO-C_6H_4
C_3	4-Cl-C_6H_4
C_4	4-F-C_6H_4
C_5	2-furyl
C_6	2-thienyl

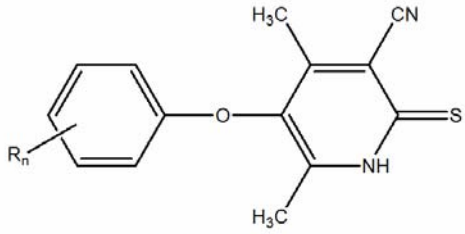
Compd. No	R
D_1	HCONHPh
D_2	HCSNHPh

Ar_2 =



B.I =





Structue (E)

Compd. No	R_n
E_1	H
E_2	4-Cl
E_3	2-Br
E_4	2-CHO
E_5	2,4-Cl_2
E_6	3-Me, 4-NO_2
E_7	2,4,6-Cl_3

3- *In vitro* test.

Food-poison-technique was used to evaluate the effect of the investigated compounds on the mycelial linear growth of the three tested fungi. Fifty milliliters of the aforementioned medium were poured into 150 ml conical flasks and autoclaved at 121^{o}C for 20 min. Three drops of 10% lactic acid were added to prevent bacterial contamination. Stock solution for each of the tested compound was prepared (v/v) by dissolving the appropriate amount of compound in 10 ml dimethyl sulfoxide (DMSO). Different volumes of each compound were added to sterile molten (40^{o}C) PDA to get a series of concentrations ranged from 125ppm to 1000 ppm for each compound in PDA. A zero (o) concentration treatment was prepared for each fungus which contained 1ml of the solvent to ensure an equivalent concentration in all treatments. Compounds-amended PDA was dispensed aseptically into 9 cm diameter Petri dishes (Tremblay *et al.,* 2003).

Plugs of mycelium (4 mm diameter) were cut from the margins of actively growing cultures of the *R. solani*, *R. tulasnea* and *S. rolfsii* fungi and placed in the center of compound-amended and un amended PDA plates with 4 replicates for each fungus-compound combination. All plates were incubated at 25 ± 1^{o}C. Colony diameter was measured for all fungi after complete growth of control and the percentage of growth inhibition was calculated for each compound. The estimated effective concentration (EC_{50}), toxicity index (T.I) for each compound under investigation were determined.

RESULTS AND DISCUSSION

1- Preliminary evaluation of the fungicidal activity of the synthesized compounds on the tested fungi.

Data in table (1) represent the antifungal activities of the new pyridinethione derivatives on *S. rolfesii, R. solani* and *R. tulasnea* fungi linear growth inhibition. Different degrees of mycelial growth inhibition were observed for fungi treated by the concentration of 1000 ppm for each compound.

The fungicidal potencies of the investigated compounds on the three fungi were varied depending on the two main factors,i.e.fungi sensitivity to the prepared chemical compound and the substitution on the pyridinethione ring.

(A) Sensitivity of the studied fungi to the prepared compounds.

Depending on obtained data *R. solani* was the most sensitive fungi to the investigated compounds followed by *S.rolfsii* and *R. tulasnea, R. solani* affected by twenty compounds, from the twenty three studied compounds, with different degrees of inhibition. On the other hand compounds that recorded inhibition effect against *R.solani* ranged between (60-98.9%) were considered as highly effective compounds were as that recorded inhibition percentages ranged between (36.4-47.5%) were considered as moderat effectivenesse compounds.The other compounds were considered as slight effective compounds .

(B) Substitution on the pyridinethione ring system.

Data in the same table showed the variation of the fungi mycelial growth in relation to the substitution on pyridinethione ring especially on position number 5 of the ring. It is clear that, introducing the benzimidazole nucleus in position 5 of the ring parallel with the presence of halogen atoms on aryl group of position 4 of the ring increased the biological activity as found in compounds C_3 and C_4. Also, introducing the aryloxy group in position 5 in the pyridinethione ring increased its activity as in structure E especially when this aryloxy group includes bromo or formyl or methyl or nitro groups. This was increased the ring activity against the three fungi as in compounds E_3, E_4 and E_6. In contrast, treatment of substituted pyridinethiones upon Thorpe zigler reaction to afford the thieno[2,3-b]pyridines] decreased the ring activity as in compounds B_1, B_2 and D_1.

Generally, any compound possessed an inhibition percent greater than 50% was considered to be promising and should be rescreened with a serial of concentrations ranged from 125-1000 ppm to calculate its EC_{50} values. Accordingly, most potent compound will be formulated and be used as a local fungicide after carrying out the required studies in the future including risk assessment.

Table (1) Fungicidal activities of the new synthesized compounds at 1000ppm against the three tested fungi

tested Compounds	*MEAN % OF THE MYCELIAL GROWTH INHIBITION UPON TREATED BY 1000 PPM*		
	S. ROLFESII	**R. SOLANI**	**R. TULASNEA**
A_1	28.6*	36.4	00.0
A_2	21.9	44.2	00.0
A_3	68.9	24.2	00.0
A_4	39.2	42.4	00.0
A_5	24.8	12.7	00.0
A_6	00.0	60.0	21.1
B_1	00.0	79.6	00.0
B_2	00.0	00.0	00.0
C_1	00.0	27.8	00.0
C_2	00.0	43.3	00.0
C_3	55.6	81.2	00.0
C_4	97.5	85.5	00.0
C_5	48.1	18.9	00.0
C_6	62.2	25.9	00.0
D_1	00.0	83.9	00.0
D_2	70.0	43.7	00.0
E_1	00.0	47.6	00.0
E_2	00.0	82.0	00.0
E_3	78.5	76.0	45.5
E_4	00.0	86.7	37.8
E_5	00.0	00.0	00.0
E_6	99.6	98.9	99.9
E_7	00.0	00.0	00.0

*Each number represent the average of four replicates.

2- Fungicidal activities of the promising synthesized compounds against the three tested fungi.

Data in table (2) represent the fungicidal activities of the promising compounds against the three tested fungi. It is clear that, compound E_6 is the most effective one on *S. rolfesii* fungus with value is the lowest EC_{50} 211.3 ppm with the toxicity index of 100.0. In contrast, compound C_3 was found to be the least effective showingEC_{50} and toxicity index of 857.6 ppm and 24.1, respectively.

Data in the same table showed that, pyridinethione derivative C_4 followed by E_6, E_4, D_1 and C_3 proved the highest effective derivatives on *R. solani* fungus, showing EC_{50} values of 184.4, 230.6, 243.9, 249.2 and 251.6 ppm, respectively. Also, it was found that, compound E_6 was the only pyridinthione derivative that affects significantly the growth of *R. tulasnea* fungus with EC_{50} value is 211.3 ppm.

Table (2):- Fungicidal activities of the promising compounds on the three targeted fungi

FUNGI	used Compounds	% of mycelial growth inhibition					EC_{50} (ppm)	Slope	Toxicity index
		1000 ppm	750 ppm	500 ppm	250 ppm	125 ppm			
S. rolfesii	C_3	55.6	46.0	30.9	19.0	10.4	857.6	1.50	24.1
	C_4	97.5	90.3	46.9	12.5	0.3	473.5	5.10	44.6
	E_3	78.5	68.9	48.5	41.0	25.0	341.2	1.48	61.8
	E_6	94.0	88.5	86.5	69.2	54.0	211.1	3.90	100.0
R. solani	B_1	79.0	70.0	59.5	39.0	24.0	365.3	1.67	50.5
	C_3	81.2	77.0	75.2	58.4	45.6	251.6	2.30	73.2
	C_4	85.5	80.3	67.1	50.5	38.0	184.4	1.50	100.0
	D_1	83.9	78.0	61.8	49.3	30.5	249.2	1.64	73.9
	E_2	82.0	73.0	54.0	38.0	20.7	335.8	1.90	54.9
	E_3	76.0	68.0	48.0	32.0	0.2	428.4	1.90	43.0
	E_4	86.7	81.1	65.6	21.1	9.2	243.9	1.76	75.6
	E_6	98.9	94.0	80.6	55.1	16.9	230.6	3.6	80.2
R. tulasnea	E_6	99.94	98.6	90.0	43.8	11.6	211.3	4.80	-

- EC_{50}: is the effective concentration that inhibits 50% of the fungal mycelial growth.
- Toxicity index: is the % of the estimated EC_{50} value with respect to the most potent one whose toxicity index was taken to be 100.0.(E6).

On the other hand, derivatives C3 against *S.rolfsii* and C4 against *R.solani* possessed the same slope values 1.5. Also, the same was recorded with E2 and E3 against *R.solani* slope values,reaching 1.9. ItCould be concluded that C3and C4 prodacts as well as E2 and E3 may be act on tested fungi by same mode of action.

3- Preparation of E_6 compound in 20% EC formulation.

Pesticide formulation is a mixture of the biologically active substance, namely active ingredient (a.i), with other adjuvants to obtain the end product to be used in pest control operation. Initially, there are many types of formulations, their efficacies and performances depends on the physical and chemical characteristics offthe active ingredient..Formulation amid to actieve the delivering the active ingredient to the site where the pathogen is. The main reason for making pesticide formulation is to facilitate the process of handling, storage and application of active ingredient much easier, safer and accurate (Knowles, 1998).

3.1. Components of the prepared formulation

Active ingredient (compound E6)	20.00%
ionic surfactant (potassium lurate)	4.66%
Non ionic surfactant (tween 80)	6.66%
Solvent 1 (n-butanol)	8.68%
Solvent 2 (dimethylformamide)	60.00%
Total	100.00%

3.2- Physico-chemical properties of the prepared formation spray solution.

Data in table (3) represent the physico-chemical properties of the local formulation in the three tested types of water (tapT.W, soft S.w and hard H.W). From the table, it was clear that the values of the seven measured parameters were varied according to the type of water used in dilution. The obtained results showed that the prepared formulation passed successfully the standard tests.

Table (3): Physico-chemical properties of the formulation spray solution

Test (unit)	T. W.	s. W.	H. W.
1- Conductivity (m mh0s)	250.0	300.0	800.0
2- Salinity (%)	0.0	1.0	2.0
3- Surface tension (dyne/cm)	29.3	30.0	31.3
4- pH value	5.80	6.07	6.37
5- Viscosity (centipoises)	8.2	7.9	7.2
6- Foam (cm)	0.0	0.0	0.0
7- Emulsion stability	√	√	√

4- Fungicidal activity of the local EC formulation against the three targeted fungi.

Data in table (4) showed the fungicide activity of the local (20% EC) formulation on the three tested fungi as compared with the commercial fungicide Moncut 25% wp (flutolanil)for *R.solani* and *S.rolfsii* and Ridomil gold plus 42.5% wp (mixture of copper oxychloride and Mefenoxam) for *R.tulasnea* . From the data it was found that, *S. rolfesii* fungus has higher susceptibility against the new formulation than compared with the commercial fungicide. Whereas, the highest concentrations of the two commercial fungicides were found not effective against the fungus. So, its EC_{50} could not be calculated but the new formulated product was found to be more effective,showing the EC_{50} value of 150.0 ppm.

Also, the local formulated product was found to have good efficacy on *R.. tulasnea* fungus and to some extent more than the commerical fungicide Ridomil gold plus 42.5% wp. The EC_{50} values of the new formulation and Ridomil gold plus recorded 97.0 and 105.5 ppm, respectively. In contrast, *R. solani* fungus was found to be very susceptible to the Moncut 25% wp than the new formulated compound where even at the lowest concentration the fungicide produced a complete inhibition for the fungal mycelial growth.

Table (4): The percentage of mycelial growth inhibition of the tested formulated products on the three studied fungi.

Conc. (ppm)	% of mycelial growth inhibition						
	S. rolfesii		***R. solani***		***R. tulasnea***		
	A	**B**	**A**	**B**	**Conc.**	**A**	**C**
200	69.2	00.0	51.2	100.0	500	89.3	80.0
100	32.6	00.0	47.0	100.0	400	86.7	72.2
75	9.7	00.0	45.0	100.0	300	80.3	70.0
50	2.3	00.0	41.0	100.0	200	71.6	57.7
25	0.0	00.0	33.o	100.0	100	51.2	49.3
EC_{50}	150.0	**	155.0	**	EC_{50}	97.0	105.5
Slope	4.42	**	1.39	**	Slope	1.8	1.6

- A is the local formulated product
- B is the commercial fungicide Moncut 25% wp.
- C is the commercial fungicide Ridomil gold plus 42.5% wp.
- ** These values can not be calculated.

REFERENCES

Arena, H. C., V. Bruggen, and P. A. Arneson (1984). Resistance in *Rhizoctonia solani* to tolclofos-methyl. European journal of plant pathology 90,95

Ibrahim, A. N (2008). Discovery of some benzimidazole Derivatives as a new Agrochemical Fungicides, Egypt. J. Chem., 51, 823.

Knowles, D. A. (1998). Chemistry and technology of agricultural formulation. Kluwer Academic , London.

Madkour, H. M. F., A. A. Farag, S. Sh. Ramses, and N. A. Ibrahim (2006). Synthesis and fungicidal activity of new imidazoles from 2-(chloromethyl)-1H-benzimidazole. Phosphorus, Sulfur and Silicon, 181, 255.

Mohamed, G. G., N. A. Ibrahim and Attia A. E. Hanaa (2009). Synthesis and antifungal activity of some transition metal Complexes with benzimidazole dithiocarbamate ligand. Spectrochimica Acta part A 72, 610.

Shim, M. y, J. L. Starr and N. P. Keller (1998). Distribution of isolates of *Sclerrotium rolfesii* tolerant to pentachloronitrobenzene in Texas peanut fields. Plant Dis. 82, 103.

Sullia, S. B. and Rose Maria. (1985). Acquired cyclohexamide resistance in *Neurospora crassa* and *Sclerrotium rolfesii*. Proc. Plant Science, 95, 417.

Tremblay, D. M., B. G. Talbot; and O. Carisse (2003). Sensitivity of botrytis squamosa to different classes of fungicides. Plant Dis., 87:570

Youssef, M. A., N. A. Ibrahim, A. E. H. Attia, and S. E. S. Hamoda (2008). Synthesis and formulation of some new azines and evaluation of their fungicidal efficiency. Egypt. J. Chem., 51, 163.

التأثيرات البيولوجية لبعض مشتقات البيريدين ثيون الجديدة المجهزة كمبيدات فطرية

ناصر عبدالمنعم إبراهيم و هناء عبدالباقى عيد عطية و سعد العدوى حمودة

المعمل المركزى للمبيدات - مركز البحوث الزراعية - الدقى- جيزة – مصر

فى هذة الدراسة تم إجراء التقييم الحيوى المعملى لعدد ثلاثة وعشرون مشتقاً جديداً لمركب البيريدين ثيون كمضادات فطرية على ثلاثة أنواع من الفطريات و هم *S. rolfesii, R. solani, R.. tulasnea* وقد وجد أن هناك علاقة مابين المجاميع والذرات المستبدلة ومواقع أستبدالها على حلقة البيريدين ثيون وبين الكفاءة الإبادية للمشتقات الناتجة عن هذة الآستبدالات على الفطريات محل الدراسة حيث وجد أن أدخال بعض المجموعات الفعالة على الحلقة زاد من كفاءتها زيادة كبيرة. تم تجهيز أكثر المركبات كفاءة فى صورة مستحضر مركز قابل للأستحلاب (20% EC) وبقياس الخواص الطبيعية للمستحضر الجديد وجد أنها تتوافق مع المعايير الدولية المحددة لذلك. تم تقييم المستحضر الجديد معملياً على نفس الفطريات ومقارنة فعاليتة بفعالية المبيدان التجاريان ريدوميل جولد بلاس 42.5% w.p (مبيد تجارى على فطر *R tulasnea* .) و المبيد مون كت 25% w.p (مبيد تجارى على*S. rolfesii, R. solani*). دلت النتائج المتحصل عليها أن المستحضر الجديد قد أعطى كفاءة تثبيطية أعلى من المبيدات القياسية على بعض الفطريات محل الدراسة. (*R.tulasnea* and *S.rolfsii*)