Phytocidal effects of some azole derivatives

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ABSTRACT

One, two and three nitrogen atoms containing five-memberred heterocyclic derivatives were tested for their phytocidal effects against seed germination, seedling root and shoot systems growth of monocotyledonous (Triticum aestivum L.) and dicotyledonous (Cucurbita pepo) plants. Six benzotriazole, six indole and four pyrazole derivatives were used. Phytocidal effects of the tested compounds appeared as a function of chemical structure. Structure activity relationships were illustrated using different concentrations of the tested derivatives. All of the tested compounds affected the seedling growth more than germination process when treated as pre-emergants. Some stunning effects on wheat primary root and malformations in squash roots were noticed. Substitution with 5,6-dichloro-moiety in 5,6-dichlorobenzotriazole (3) highly increased the phytotoxicity against both seed germination and seedling growth with EC₅₀ values equalled 10.5 and 5.0 μg/ml, respectively. The caused strong inhibition of squash root and shoot systems with (8.6 and 16.8 µg/ml), while those of wheat seedlings (3.4 and 12.4 µg/ml) exceeding that of the standard herbicide. Some other tested derivatives were more potent than the standard herbicide in their effects. 3-Methyl-1(2,4-dinitrophenyl) pyrazol-5-one (17) showed the highest of pyrazole derivative for squash seedlings with EC50 values of 47.2 and 77.1 µg/ml, respectively. Both compounds 3 and 17 inhibited both fresh and dry weights of wheat seedlings as concentration increased with reduction percentages reached 94.2%. They affected the sugars contents as a function of concentration and time after treatment. The most active concentration was 100 $\mu g/ml$ in reducing chlorophyll a and b as well as soluble phenolic compounds.

Keywords: Phytocidal activity, benzotriazole, indole, pyrazole, chlorophyll, soluble phenolics, sugars

INTRODUCTION

Nitrogen containing heterocyclic copounds are used as insecticides, herbicides and fungicides or plant growth regulators as some 1,2,4-triazoles (Quinn et al., 1985). They also reported that nitrobenzotriazole derivatives were known to exhibit good antifungal effects against several species comparable to fluconazole and clotrimazole, while bromobenzotriazole derivatives showed good antibacterial activity against Bacillus subtilis. In addition, Ahmed et al. (2004) stated that potent fungicidal activity of 5,6-dichlorobenzo-triazole inducing several biochemical effects. Robin (1986) reported that benzotriazole induced male sterility in wheat, reducing grain yield when applied to the soil at sowing or at late tillering to either soil or foliage.

Coordination of copper, zinc, silver and cobalt with benzotriazole, 5methylbenzotriazole and 2-methylbenzotriazole showed herbicidal capacity on both monocotyledons and dicotyledons (Caramazza et al., 1990). Triazolinones displayed potential herbicidal activities with promising candidates comparing with the commercial product sulfentrazone (Luo et al., 2008). Amitrole (ATz, 3-amino-1H-1,2,4-triazole) is a widely employed herbicide (Watanabe et al., 2005).1-Aminobenzo-triazole (ABT). the P₄₅₀ inhibitor synergized 5-ketoclomazone against water grass, Echinochloa phyllopogon (Stapf.) Koss. that is resistant to multiple herbicides reducing chlorophyll and carotenoids contents (Yassour et al., 2008). Indole derivatives as indol-3-acetic acid (IAA), its 5-methoxy derivative and 1H-indole-4,7-diones reduced spore germination, mycelial dry weight and protein content of several fungi (Sharaf and Farrag, 2004; Kumar et al., 2007 and Ryu et al., 2007). Abdel-Aty (2010) reported that 2phenylindole and 1-acetylindole-3-butyric acid exhibited persuasive fungicidal activity affecting polyphenoloxidase (PPO), peroxidase (PO), DNA and RNA contents and the fungal sugar contents in vivo developing deformed and dead cells. Although IAA usually stimulates growth and developmental processes, at high concentrations it induced growth abnormalities including leaf epinasty, growth inhibition of root and shoot, chloroplast damage, and destruction of membrane and vascular system integrity, leading to desiccation, tissue necrosis, and decay (Grossman, 2003). Pyrazole derivatives showed systemic herbicidal effects as pyrazoxyfen, pyrazosulfuron-ethyl and pyrazolynate that are absorbed through the young stems and roots of weeds. 3-methyl-1-(2,4-dinitrophenyl)-

pyrazol-5-one *in vitro* inhibited the mycelial growth of *Coriolus versicolor* and *Gloeophyllum trabeum* with decreasing the wood mass loss (Abdel-Aty and Mohareb (2008). Phytocidal activities of certain pyrazole derivatives have been reported by Abdel-Aty (2007).

So, this study was directed to evaluate some five-membered heterocyclic compounds containing one atom (indole), two atoms (pyrazole) and three atoms (benzotriazole) of nitrogen for their phytocidal effects against seed germination, seedling root and shoot systems growth and dry weight. The biochemical effects of the active derivatives on chlorophyll content, soluble phenolics and sugar content in wheat plants as toxicity parameters are also studied (Oncel et al., 2000) to determine their most useful use.

MATERIALS AND METHODS

- 1. Chemicals: Indole-3-acetic acid (7), El-Gomhouria Drug Company: indole-3-butyric acid (8), Sisco Research Laboratories, Mumbai, India, and other chemicals and solvents were obtained from El-Gomhouria Drug Company, Egypt. The standard metribuzin (sencor), (4-amino-6-tert.butyl-4.5-dihydro-3-methylthio-1,2,4-triazin-5-one) was donated by Kafr El-Zayat Company for pesticides, Egypt. Determination of soluble sugars, chlorophyll contents and total soluble phenols (TSP) were done on Unico-1200 Spectrophotometer.
- 2. **Tested compounds**: Benzotriazole derivatives: benzotriazole (1), 5.6-dimethylbenzotriazole (2), 5.6-dichlorobenzotriazole (3), 1-acetyl-5.6-dimethylbenzotriazole (4) 1-benzoylbenzotriazole (5) and 1-benzoyl-5.6-dimethylbenzotriazole (6) were previously prepared (Abdel-Aty, 1996). Besides the purchased indole-3-acetic acid (7) and indole-3-butyric acid (8), 1-acetylindole-3-butyric acid (9), 1-benzoylindole-3-acetic acid (10), 1-benzoylindole-3-butyric acid (11), 2-phenylindole (12), 1-acetyl-2-phenylindole (13) and 1-benzoyl-2-phenylindole (14) were prepared (Abdel-Aty, 2010). Pyrazole derivatives: 3.5-dimethylpyrazole (15), 1-benzoyl-3,5-dimethylpyrazole (16), 3-methyl-1-phenylpyrazol-5-one (17) and 3-methyl-1-(2,4-dinitrophenyl)pyrazol-5-one (18) were prepared (Abdel-Aty (2007). Metribuzin (sencor), (4-amino-6-tert.butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) was used for data comparison

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as a standard herbicide. Chemical structures of the tested derivatives are shown in Figure (1).

| R ₂ | N |
|----------------|----|
| R ₃ | |
| 3 | R, |

| Compound | R, | R ₂ | R_3 |
|---|----------------------------------|-----------------|-----------------|
| Benzotriazole (1) | Н | Н | Н |
| 5.6-Dimethylbenzotriazole (2) | Н | CH ₃ | CH_3 |
| 5.6-Dichlorobenzotriazole (3) | Н | Cl | Cl |
| 1-Acetyl-5.6-dimethylbenzotriazole (4) | CH ₃ CO | CH ₃ | CH ₃ |
| 1-Benzoylbenzotriazole (5) | C ₆ H ₅ CO | Н | Н |
| 1-Benzoyl-5.6-dimethylbenzotriazole (6) | C ₆ H ₅ CO | CH ₃ | CH ₃ |

| | | | 00 000 00 00 00 00 00 00 00 00 00 00 00 |
|-------------------------------------|----------------------------------|-------------------------------|---|
| Compound | R ₁ | R_2 | R_3 |
| Indole-3-acetic acid (7) | Н | Н | -CH ₂ -COOH |
| Indol-3-butyric acid (8) | Н | Н | -(CH ₂) ₂ -COOH |
| 1-Acetylindole-3-butyric acid (9) | CH₃CO | Н | -(CH ₂) ₂ -COOH |
| 1-Benzoylindole-3-acetic acid (10) | C ₆ H ₅ CO | Н | -CH ₂ -COOH |
| 1-Benzoylindole-3-butyric acid (11) | C ₆ H ₅ CO | Н | -(CH ₂) ₂ -COOH |
| 2-Phenylindole (12) | Н | C_6H_5 | Н |
| 1-Acetyl-2-phenylindole (13) | CH ₃ CO | C_6H_5 | Н |
| 1-Benzoyl-2-phenylindole (14) | C ₆ H ₅ CO | C ₆ H ₅ | Н |

| N. | 1 | | |
|--|---|-----------------|-----------------|
| Compound | R_1 | R_2 | \mathbb{R}_3 |
| 3.5-Dimethylpyrazole (15) | Н | CH ₃ | CH ₃ |
| 1-Benzoyl-3,5-dimethylpyrazole (16) | C ₆ H ₅ CO | CH_3 | CH_3 |
| 3-Methyl-1-phenylpyrazol-5-one (17) | C ₆ H ₅ | CH_3 | =O |
| 3-Methyl-1-(2.4-dinitro-phenyl) pyrazol-5- one (18) | (NO ₂) ₂ - C ₆ H ₃ - | CH ₃ | =O |

Fig. (1). Chemical structure of the tested benzotriazole and indole derivatives

3. Phytocidal effects

- 3.1. Cotton plug technique test: The tested compounds were checked for their phytotoxicity on wheat seeds using the cotton plug technique (Grodzinsky and Grodzinsky, 1973) at 50, 100, 200, 500 and $1000 \mu g/ml$. They were dissolved in dimethylsulfoxide (DMSO) at a concentration as high as 1% of the tested solution volume. Thirty wheat seeds were used in each replicate. Three replicates were considered as a treatment. Control was concurrently conducted. After 10 days, number of germinated seeds was counted and the height of the grown seedlings was measured.
- 3.2. Plain agar test: Phytocidal effects were tested against wheat seedlings (*Triticum aestivum*) and squash seedlings (*Cucurbita pepo*) using the plain agar technique (Zemanek, 1963). The tested compounds were mixed with the plain agar medium at 50, 100, 200, 500, 1000 and 2000 μ g/ml and poured into test tubes. Pre-germinated seeds were sown in the solidified agar. Three replicates were used for each treatment. Control was concurrently done at the same conditions. The test tubes were watered with a constant volume until roots reached the bottom of a tube. The length of both root and shoot systems were measured and inhibition percentages were calculated and the obtained EC_{50} values were compared with that of the standard herbicide.
- 3.3. Effect on dry weight: In pre emergence treatment with tested compounds at 2, 5, 10, 15, 20, 30, 50 and 100 µg/ml, the grown wheat plants were collected at soil surface and weighed. The fresh weight was dried at 105 °C to a constant weight and the resultant dry weight was recorded. In the post emergence treatment, 1.0 g fresh weight was heated at 105 °C for a constant time (1 hour) in all treatments to remove the water content and the remained dry matter was compared at different concentrations and exposure times. Dry matter content (DMC) was determined in both cases.
- 3.4. Biochemical effects: Grown wheat plants (36 days old) were treated once with 5.6-dichlorobenzotriazole or 3-methyl-1-(2,4-dinitrophenyl) pyrazol-5-one at 5.10, 20, 50, 100 and 200 μ g/ml using overall foliar application with a hand sprayer. The tested compounds were dissolved in DMSO at a concentration as high as 0.4 % in volume of the used final

aqueous solution. Samples were taken randomly after 0, 3, 7, 15 and 25 days and used for determination the followings:

- 3.4.1. Effect on sugar contents: Dried wheat seedlings (0.5 g) were blended with 10 ml of 80% aq. EtOH and 1 ml aliquot of the resulted extract was used for determining total soluble sugars (T.S.S), reducing sugars (R.S) and non-reducing sugars (non-R.S) expressed as $\mu g/g$ dried plant using the picric acid colorimetric method at 540 nm (Thomas and Dutcher, 1924).
- 3.4.2. Effect on chlorophyll contents: Fresh leaf tissues (50 mg) were dipped in 3.0 ml of EtOH and incubated in the dark for 2 hrs. Each sample was homogenized and centrifuged at 13000 rpm for 10 min. Chlorophyll (a and b) contents were calculated in $\mu g/g$ tissue fresh weight at 650 and 665 nm against EtOH (Hipkins and Baker, 1986).
- 3.4.3. Effect on total soluble phenols (TSP): Leaf tissue (0.255 g) was frozen in 7 ml of 95% aq. EtOH for 48-72 hrs and homogenized; and then 0.5 ml of the resulted filtrate was mixed with 95% EtOH (0.5 ml), H₂O (2.5 ml) and folin ciocalteu's phenol reagent (0.25 ml) in a test tube. After 5 min, 1.0 ml of 5% Na₂CO₃ was strongly shaken with the mixture and incubated at room temperature for 1 hr in the dark. Total soluble phenolics were determined spectrophotometrically at 725 nm and calculated as mg gallic acid equivalent (mg GAE)/g fresh weight (McCue et al., 2000 and Horii et al., 2007).

4. Statistical analysis

Inhibition percentages in germination and seedling heights were recorded and the EC_{50} values were calculated using Costat software (1986). The obtained results were compared with that of the used standard herbicide.

RESULTS AND DISCUSSION

1. Phytocidal effects

1.1. Seed treatment (cotton plug technique)

Phytocidal effects of the tested compounds on wheat seeds are recorded in Table (1) as effective concentration inhibiting 50% of seed germination or seedling growth (ED₅₀) values. Metribuzin (Sencor) as standard herbicide)

Table (1). Phytocidal effects of the tested compounds using cotton plug technique.

| tech | nique. | | | | | | | | | |
|--|------------------------------|-----------------|-----------------|--------------|-------------------------------|-------------------------------|------|------|--|--|
| W 12 12 12 12 12 12 12 12 12 12 12 12 12 | Inhib | ition of seed | d germin | ation | Inhibit | Inhibition of seedling growth | | | | |
| Tested compound | EC ₅₀ (95% CL) | Slope ± SE | | ΥF | EC ₅₀ (95% CL) | Slope ± SE | χ² | TF | | |
| Be nzotriazole derivativ es | | | | | 1 | - 52 | | | | |
| Benzotri azole (1) | 324 (268–391) | 1.54± 0.020 | 4.5 | 3.05 | (94 - 130) | 1.97± 0.028 | 10.4 | 7.24 | | |
| 5.6- Dimethylbenzotriazole (2) | 390 (309–495) | 1.27± 0.018 | 7.4 | 3.67 | 77.1 (61 – 97.2) | 1.56 ± | 2.8 | 5.07 | | |
| 5.6- Dichlorobenzotriazole (3) | 10.5 (9.6–11.4) | 4.32± 0.105 | 1.8 | 0.099 | 5.0 (4.1~6.1) | 2.58 ± 0.074 | -1 | 0.33 | | |
| 1-Acetyl-5,6- dimethylbenzotriazole (4) | > 1000 | = | | 170 | 156.6 (128–192) | 1.39 ± 0.019 | 191 | 10.3 | | |
| 1-Benzoylbenzotriazole (5) | > 1000 | -4 | === | <u>***</u> * | 307 (206 – 461) | 0.67 ± 0.015 | 0.7 | 20.2 | | |
| l-Benzoyl-5,6- dimethylbenzotriazole (6) | > 1000 | - | 20 | =1 | 368.9 (293 –464) | 1.28 ± 0.018 | 2.8 | 24.3 | | |
| indole derivatives | | 26 | 49 4000 400 | 78 N - 10 | | 60101110010 | 307 | | | |
| Indole-3-acetic acid (7) | 378.2 (319 – 448) | 1.8 ± 0.023 | 5.7 | 3.56 | 75.4 (52 - 110) | 0.95± 0.018 | 1.8 | 4 96 | | |
| Indol-3-butyric acid (8) | 502.5 (430 – 588) | 2.2 ± 0.034 | 3.2 | 4.73 | 99 0 (83–118) | 1.88 ± 0.028 | 12.6 | 6.50 | | |
| I-Acetylindole-3- butyric acid (9) I-Benzoylindole-3- | 97.4 (78 – 122) 495.8 | 1.45± 0.022 | 15. 8 | 0.92 | 33.5 (22.7–49) | 1.58± 0.043 | 46 | 2.20 | | |
| acetic acid (10) I-Benzoylindole-3- | (392 -628) | 1.39± 0.020 | 2.2 | 4.66 | 209 6 (167 – 263) | 1.17± 0.017 | 2.8 | 13.8 | | |
| butyric acid (11) | > 1000 | (-0) | _% | -2 | 401.9 (335 – 482) | 1.71 ± 0.023 | 0.5 | 26.4 | | |
| 2-Phenylindole (12) I-Acetyl-2-phenylindole | > 1000 | - | 93 8 | n= | 212.7 (181–250) | 1.79 ± 0.022 | 19 | 14.0 | | |
| (13) I-Benzoy1-2- | > 1000 | 2.44± | () | - | 339.6 (289 – 399) 109.1 | 1 89 ± 0 024 | 14.8 | 22.3 | | |
| phenylindole (14) Pyrazole derivatives | (267 –346) | 0.032 | 5.1 | 2.86 | (97 –123) | 2.83± 0.055 | 13.4 | 7 2 | | |
| 3.5-Dimethylpyrazole (15) | 831.8 (652 – 1062) | 1.74± 0.032 | 2.7 | 7 83 | 159.1 (136 – 186) | 1.91 ± 0.024 | Į Į | 10.5 | | |
| 1-Benzoyl-3,5- dimethylpyrazole (16) | 822.8 (651- 1041) | 1.8± 0.033 | 2.7 | 7.74 | 213.7 (183 – 249) | 1.88± 0.023 | 1.8 | 14 . | | |
| 3-Methyl-1- phenyipyrazol-5-one (17) 3-Methyl-1-(2,4-dinitro- | 564 (462 –690) | 1.75± 0.026 | 8.3 | 5.31 | 195 (159 – 240) | 1.32 ± 0.018 | 4.3 | 12.8 | | |
| phenyl) pyrazol-5-one (18) | > 1000 | | <u></u> | - | 172.4 (118– 249) | 0.71± 0.015 | 6.8 | I1 4 | | |
| Sencor (Metribuzin) | 106.3 (85.3 -132) | 1.43± 0.021 | 18. 6 | 0.1 | 15.2 (13 -18) | 2.08± 0.062 | 2.1 | 1 1) | | |

TF. toxicity factor related to metribuzin: Degree of Freedom = 3; EC_{50} and 95% Confidence limit (95% CL) are in μ g/ml

inhibited seed germination and the emerged seedling growth (length) with EC₅₀ values equalled 106.3 and 15.2 µg/ml, respectively.

Growth of wheat seedlings was found more sensitive to all tested compounds than seed germination process. Pyrazole derivatives were less effective than both indole and benzotriazole derivatives against the seed germination, while their effects against vegetation was depending on the derivative structure. 5,6-Dichlorolbenzotriazole was more potent than the standard herbicide, metribuzin against both seed germination and growth of seedlings with EC50 values equalled 10.5 and 5.0 µg/ml, respectively. 1-Acetylindole-3-butyric acid caused nearly the same effect of metribuzin on seed germination with EC50 value equalled 97.4 µg/ml, whereas its effect on seedling growth was less than it with 33.5 µg/ml (half effect of the standard herbicide). However, the other tested benzotriazoles, indole and pyrazole derivatives were less effective against both seed germination and seedling growth in comparison to the standard herbicide.

1.2. Effect on seedling stage (Plain agar technique): Screening effects of the tested compounds on root and shoot systems of quash (C. peppo) and wheat (T. aestivum) are shown in Tables 2 and 3. The standard herbicide inhibited the growth of squash root and shoot systems with EC50 values equalled 86.2 and 97.2 µg/ml, respectively. 5,6-Dichlorolbenzotriazole caused very strong effect to inhibit root and shoot system with EC50 values equalled 8.6 and 16.8 µg/ml, exceeding the standard herbicide. 1-Acetyl-5.6-dimethylbenzotriazole and 3-methyl-1(2,4-dinitrophenyl) pyrazol-5-one were also more potent than sencor, since they caused inhibition with EC50 values equalled 26.2 and 47.2 µg/ml, respectively and with 72.2 and 77.1 µg/ml respectively against root and shoot systems of squash seedlings. 5,6-Dimethylbenzotriazole, 1-benzoylbenzotriazole and 1-acetylindole-3butyric acid caused higher inhibition more than sencor against squash root system with 63.1, 71.2, and 80.1 µg/ml EC50 values, respectively. Indole-3butyric acid inhibited squash shoot system with EC50 value equalled 63.7 μg/ml. The other tested benzotriazoles, indole and pyrazole derivatives were less effective than the standard herbicide against root and shoot systems of squash seedlings.

Table (2). Phytocidal effects of the tested compounds on squash (C. Peppo) seedlings.

| | | tion of roc | | Inhibition of shoot growth | | | | |
|--|------------------------------|----------------|-----------|----------------------------|-----------------------------|---------------------------|-----------|----------|
| Tested compound | EC ₂₀ (95%-CL) | Slope ± SE | χ² | TF | EC50 (95% CL) | Slope ± SE | χ² | TF |
| Benzotriazole | | | | | _ | | | |
| derivatives | | | | | | | | |
| Benzotriazole (1) | 88 3 (74.2-105) | 1.98± 0.029 | 4.06 | 1.02 | 154 3 (133-179) | 1.98± 0.020 | 2.24 | 1.59 |
| 5,6- Di methylbenzot riazole | 63.1 (54-73.8) | 2.72± 0.085 | 1 79 | 0.73 | 99 <u>2</u> (89.6-109 8) | 3 66± 0 110 | 3.22 | 1 03 |
| (2) | (34-73 6) | 0.063 | | | (89.0-1(/9.8) | WIII | | |
| 5,6- | 8.6 | 1.02 | | | 1. 0 | 23= | | |
| Dichlorobenzotriazole | | 1.83± | 1.4 | 0.10 | 168 | 50000000 | 1.63 | 0.13 |
| (3) | 7 06-10 3) | 0.025 | | | (14.7-19.2) | 0.026 | | |
| 1-Acetyl-5,6- | n wane | 27523 | | | | | | |
| imethy/benzotnazole | 26.2 | 1.33± | 8.3 | 0.30 | 72.2 | 1 69± | 8.1 | 0.74 |
| (4) | (21.6-31.8) | 0.012 | 0.2 | 0.00 | (57 8-89 9) | 0.024 | | |
| I-Benzovibenzotnazole | 71.2 | 2.01± | | | 148 9 | 194± | | |
| (5) | (58.8-86) | 0.035 | 4.0 | 0.83 | (128-173 4) | 0.020 | 3.52 | 1.53 |
| -Benzov -5,6- | (30.0-00) | 0.033 | | | (120-1/34) | 0.020 | | |
| St. Activities and Company of the Control of the Co | 97.9 | 1.43± | 4.1 | 1.14 | 186 3 | 2.04± | 3.63 | 1 92 |
| dimethylbenzotriazole | (78-122.6) | 0.016 | 4. : | Litte | (162-215.4) | 0.019 | 3.03 | 17. |
| (6) | | | | | | E E | | _ |
| indole derivatives | 527072 TO | 100 000 | | | 1222.0 | 12 10121 | | |
| ndole-3-acetic acid (7) | 3114 | 1 56± | 2.83 | 3.61 | 201 4 | 1.08± | 3.58 | 2.0 |
| | (263-369) | 0.013 | 2.00 | 2.5. | (159-256) | 0.011 | | - |
| ndol-3-butyric acid (8) | 64 4 | 2.54± | 6.36 | 0.75 | 63.7 | 1 67± | 6.20 | 0.66 |
| ndoi-5-odiyne acid (b) | (54.7-75.8) | 0.070 | 0.30 | 0.73 | (50.2-80.8) | 0.025 | 0.40 | 0.00 |
| I-Acetylindole-3- | 1 08 | 1.21= | 10 3 | 0.93 | 156.3 | 1 31± | 4.77 | 1.6 |
| outyric acid (9) | (59.9-107) | 0.014 | 103 | 0.73 | (126.4-193) | 0.012 | 4.77 | 1 0 1 |
| -Benzoylindole-3- | 150.7 | 1.35# | 2.42 | 1.75 | 349 7 | 1 23± | 4.20 | 3 60 |
| icetic acid (10) | (122-185.4) | 0.013 | 2.42 | 1.75 | $(285 \rightarrow 29)$ | 0.011 | 4.20 | 3 00 |
| -Benzovlindole-3- | 525 | 1.44± | . 30 | | 566 | 1.0± | 0.77 | - 04 |
| outyne acid (11) | (435-633) | 0.013 | 4.30 | 6.09 | (433-741) | 0.010 | 0.73 | 5.82 |
| | 160 4 | 0.87± | 700770000 | 2710003 | 832 | 121± | no resear | 1001 200 |
| 2-Phenylindole (12) | (118-218) | 0.010 | 6.50 | 1.86 | (651-1065) | 0.012 | 1.92 | 8.56 |
| -Acetyl-2-phenylindole | 594 | 1 72± | | | 1391 | 1 37± | | |
| 13) | (504-700) | 0.016 | 5 40 | 6.89 | (1064-1821) | 0.017 | 3.90 | 14.3 |
| -BenzovI-2- | 143 | 1.54± | | | 705 | 1 38± | | |
| henylindole (14) | (119-172.4) | 0.015 | 8.22 | 1.66 | (573-868) | 0.013 | 0.30 | 7 25 |
| | (117-172.4) | 0.013 | | | (3/3-808) | 0.013 | | - |
| yrazole derivatives | 04.7 | 1.76 | | | 40.4 | | | |
| 5.5-Dimethylpyrazole | 94.7 | 1 65± | 8.40 | 1.1 | 40 4 | 1.35± | 2.94 | 0.42 |
| 15) | (78-115.6) | 0.019 | 356 1/50 | 20115 | (28-58 1) | 0 022 | 3500 IX | |
| -Benzoyl-3,5- | 128.5 | 1.88± | 3.20 | 1.49 | 1134 | 1 18± | 1.99 | 1.17 |
| limethylpyrazole (16) | (109-151) | 0.020 | 5.20 | | (87.6-146.5) | 0.012 | | |
| -Methyl-1- | 101.6 | 1 28± | | | 59.5 | 4± | | |
| henvlpyrazol-5-one | (79.2-130) | 0.014 | 9.90 | 1.18 | (41.9-84) | 0.014 | 8.35 | 061 |
| 17) | (77.2-130) | 0.014 | | 708 | (41.7-84) | 0.014 | | |
| -Methyl-1-(2,4-dinitro- | 27.3 | 1.20 | | 1 | | | | |
| henyl) pyrazol-5-one | 1701-5-one 472 139± 630 055 | | 77 1 | 1 21± | 4.90 | 0.79 | | |
| 18) | (34-654) | 0.022 | | STATUTE S | (57.5-103) | 0 014 | 0.700.700 | 1550C T |
| CONTRACT CONTRACTOR NO. | | 0.00 | _0 | 1000 1000 | | | 200 10 | |
| encor (Metribuzin) | 86.2 | 131± | 7.90 | 1.0 | 97.2 | 63± | 4.01 | 1.0 |

TF, toxicity factor related to metribuzin; Degree of Freedom = 3; EC₅₀ and 95% Confidence limit (95% CL) are in μg/ml

Table (3) showed that EC₅₆ values of sencor against root and shoot systems of wheat were equalled to 55.8 and 68.2 μg/ml, respectively. 5.6-Dichlorolbenzotriazole, indole-3-butyric acid, indole-3-acetic acid. 1-acetylindole-3-butyric acid, benzotriazole, 1-benzoylindole-3-acetic acid and 3-methyl-1(2.4-dinitrophenyl)pyrazol-5-one were more potent than the used standard herbicide with 16.9, 6.0–3.3, 2.86, 1.82, 1.8 and 1.45 times respectively against root system of wheat. On the other hand, 5.6-dichlorolbenzotriazole was found more effective than metribuzin with about 5.3 times, on wheat seedlings shoot system growth. The other tested benzotriazoles, indole and pyrazole derivatives were less effective than the used standard herbicide against both root and shoot of wheat seedlings.

All the tested benzotriazole, indole and pyrazole derivatives were more effective inhibiting growth of root system than shoot growth in squash (C, peppo) seedlings except indole-3-acetic acid (7) with EC₅₀ values equalled 311.4 and 201.4 μ g/ml and 3-methyl-1-(2,4-dinitrophenyl) pyrazol-5-one (18) with EC₅₀ values equalled 47.2 and 77.1 μ g/ml, respectively. The same trend was obtained in case of wheat seedlings by benzotriazole and indole derivatives except 1-benzoylindole-3-butyric acid (11) with EC₅₀ values equalled 611 and 544 μ g/ml respectively and 1-benzoyl-2-phenylindole (14) with EC₅₀ values equalled 453 and 503 μ g/ml, respectively. However pyrazole derivatives proved to be more effective against the root than the shoot depending on inhibition degree on the chemical structure differences among the applied derivatives except 3,5-dimethylpyrazole (15) with EC₅₀ values equalled 208 and 172 μ g/ml, respectively.

However, 5.6-dichlorolbenzotriazole proved to be a good inhibitor against monocotyledons and dicotyledonous plants. In other words, the configuration structure of 5.6-dichlorolbenzotriazole as well as 5.6-dichloro substituents may be required to act good results of this compound comparing with the other derivatives. Different effects among the tested compounds may be due to frontier orbital energy level, main constituent of orbital and charge distribution in the structure as all have great influence on herbicides bioactivity. N-acetylbenzotriazole is a particularly reactive heterocyclic amide owing to the involvement of the electron pair of the amide nitrogen in the quasi aromatic five-membered ring (Staab and Rohr, 1968). Due to high phytocidal effects of both 5,6-dichlorobenzotriazole and 3-methyl-1(2,4-dinitrophenyl)pyrazol-5-one, they were applied as post treatment to study their effects against some plant active sites.

Table (3). Phytocidal effects of the tested compounds on wheat

| | Inhibitio | on of root | growth | | Inhibition of shoot growth | | | |
|--|--|-------------------------|--------------|-----------------|--|---------------|-------|------|
| Tested compound | EC ₅₀ (95% CL) | Slope ± SE | χ² | TF | EC ₅₀ (95% CL) | Slope ± SE | χ² | TF |
| Benzotriazole | | 636 | | | | | | |
| derivatives | | | |) | eg Si za aras aras | 10 67/24000 | | |
| Benzotriazole (1) | 30.6 | 2.54± | 0. | 0.55 | 141.3 | 1.69± | 8.8 | 2.07 |
| Delizoti iazole (1) | (21.6-43.3) | 0.180 | 14 | 0.55 | (119-168) | 0.017 | 0.0 | |
| 5,6- | 100 | 2.22± | 6. | 58 | 191 | 2.26± | | |
| Dimethylbenzotriazote | (86-116) | 0.033 | 32 | 1.79 | (168-219) | 0.023 | 8.86 | 2.8 |
| .2) | 10001101 | 0,050 | | ! | e exercise money. | | | |
| ↑.6- | . 54 | 1.9± | 4. | S 5000 | 12 4 | 1.56 ± | 1.40 | 0.10 |
| Dichlorobenzotriazole | (24-47) | 0.062 | 30 | 0.06 | (10.2-15) | 0.016 | 1.42 | 0.18 |
| 13) | | ğ <u>.</u> | | | | | | |
| i-Acetyl-5,6- | 82.4 | 1.78± | 6. | | 226 | 1.39± | 2 (2 | 1 11 |
| dimethylbenzotriazole | (67.6-100) | 0.024 | 81 | 1.48 | (187-273) | 0.012 | 2.63 | 3.31 |
| (4) | AND STORY SHOULD AND STORY STO | | Real Control | | Assessment seasoners | 1.43. | | |
| I-Benzoylbenzomazole | 96.1 | 1.47± | 5. | 1.72 | 296 | 1.42± | 5.51 | 4 34 |
| (5) | (77-120) | 0.016 | 56 | | (247-355) | 0.012 | | |
| 1-Benzoyl-5.6- | 148 | 1.05± | 3 | 10 10000 | 498 | 0.87± | • • • | = 20 |
| dimethylbenzotnazole | (114-193) | 0.110 | 94 | 1.48 | (373-667) | 0.010 | 2.16 | 7.30 |
| (6- | (71.1.1.2) | EMI I | | | | | _ | |
| Indole derivatives | | | 1000 | | | | | |
| Indole-3-acetic acid (7) | 17 | 1.7± | 1. | 0.30 | 83.3 | 1.45± | 6.69 | 1.22 |
| This is a decire delia | (8.8-32.6) | 0.097 | 65 | | (66-105.5) | 0.017 | | |
| Indol-3-butyric acid (8) | 9.2 | 1.62± | 0. | 0.16 | 64.3 | 1.7± | 5.22 | 0.94 |
| 00 00 00 00 00 00 00 00 00 00 00 00 00 | (2.8-29.0) | 0.017 | 54 | | (51-81) | 0.026 | | |
| 1-Acetylindole-3- | 19.5 | 1.32± | 3. | 0.35 | 63.3 | 2.0± | 5.27 | 0.93 |
| butyric acid (9) | (10.8-34.8) | 0.035 | 24 | | (51.7-44.4) | 0.040 | | |
| I-Benzoylindole-3- | 30.9 | 1.62± | 4. | 0.55 | 262 | 1.34± | 7.9 | 3.84 |
| acetic acid (10) | (20 8-45.6) | 0.042 | 37 | | (216.2-317) | 0.012 | | |
| 1-Benzoylindole-3- | 611 | 141± | 2. | 11.1 | 544 | 0.81± | 2.98 | 7.98 |
| butyric acid (11) | (502-744) | 0.013 | 10 | | (396-749) | 0.010 | | |
| 2-Phenylindole (12) | 377 | 0.65± | 2. | 6.77 | 520 | 0.86± | 6.96 | 7.62 |
| 100 U 4- | (262-544) | 0.089 | 86 | | (387-701) | 0.010 | | |
| 1-Acetyl-2- | 902 | 1.67± | 9 | 16.2 | 1316 | 1.3± | 5.34 | 19.3 |
| phenylindole (13) | (748-1088) | 0.018 | 11 | | (998-1736) | 0.015 | | |
| 1-Benzoyl-2- | 453 | 1.26± | 3. | 8.12 | 503 | 1.5± 0.013 | 1.73 | 7.38 |
| phenylindole (14) | (369-556) | 0.012 | 5.5 | 900,000,000,000 | (421-603) | 0.013 | | |
| Pyrazole derivatives | | | • • | | 170 | 1.50 | | |
| 3.5-Dimethylpyrazole | 208 | 1 68± | 12 | 3.73 | 172 | 1.58 | 6.18 | 2.52 |
| (15) | (177-245) | 0.015 | .3 | | (144-205.3) | ±0.014 | | |
| 1-Henzovl-3.5- | 106 | 1.08 ± | 6. | 1.9 | 381 | 1.76± | 7 74 | 5.59 |
| dimethylpyrazole (16) | (79.3-140.6) | 0.012 | 87 | | (326-444) | 0.015 | | |
| 3-Methyl-1- | 132 | 1.53± | 11 | 2 22 | 420 | 1.72± | 4 49 | . 14 |
| phenylpyrazol-5-one | (108.5-159) | 0.015 | .6 | 2.37 | (358.3-492) | 0.015 | 4.47 | 6.16 |
| (17) | | and recourse on the St. | | | The second section of the second section of the second section of the second section s | | | |
| 3-Methyl-1-(2.4- | 38.6 | 1.33± | 5. | 0.7 | 220 | 1.41± | 1 02 | 2.32 |
| dinitro-phenyl) pyrazol- | (26.3-56.3) | 0.022 | 16 | 0.7 | (183-265.1) | 0.013 | 11.6 | 3.23 |
| 5-one (18) | 38 38 | | | Belline No. | | 7.03 | .% | |
| Sencor (Metribuzin) | 55.8 | 2.43± | 4. | 1.0 | 68.2 | 1.82± | 5.16 | 1.0 |
| | (48.2-67.3) | 0.074 | 41 | 2,376767 | (55.1-84.3) | 0.029 | *** | |

TF, toxicity factor related to sencor; Degree of Freedom = 3; EC₅₀ and 95% Confidence limit (95% CL) are in µg/ml

1.3. Effect on Dry weight

Pre emergence treatment with 5,6-dichlorobenzotriazole inhibited both fresh and dry weights of wheat seedlings (Table 4).

Table (4). Effect of 5,6-dichlorobenzotriazole pre emergence treatment on wheat seedlings.

| | | Reduc | tion perce | nt at diffe | rent conc | entrations | (µg/ml) | | EC ₅₀ (95% CL) | Slope± | γ- | |
|--------|-----|--------------|--------------|--------------|--------------|--------------|--------------|-----|------------------------------|--|------|--|
| Weight | 0 | 2 | . 5 | 10 | 15 | 20 | 30 | 50 | | and the second s | SE | |
| Fresh | 0.0 | 45.1± 2.5 | 57 6± | 82 81 2 9 | 86 2± 3 2 | 88.4± 4.2 | 94.7± 5 l | 100 | 2.9 (2.2-3.7) | 1 59± 0.018 | 7.06 | |
| Dry | 0.0 | 30.6± 2.3 | 64.9± 2.5 | 77.9± 2 8 | 83 7± 3 6 | 90 9± 3.5 | 94.2± 4.3 | 100 | 3.6 (3.0-4.4) | 1.82± 0.021 | 4.24 | |

Fresh weight of the emerged wheat seedlings was reduced with reduction percentages ranged from 45.1 to 94.7% at concentrations ranged from 2 to 30 μg/ml respectively with EC₅₀ values equalled 2.9 μg/ml, while their dry weight was reduced increasingly with increasing the concentration, reduction percentages ranged from 30.6 to 94.2% at the same concentration range, with EC50 value equalled 3.6 µg/ml. At 50 µg/ml, it completely prevented seeds emergence. Post-emergence treatment of wheat seedlings with 5,6-dichlorobenzotriazole and 3-methyl-1-(2,4-dinitrophenyl)pyrazol-5-one affected their dry weight increase depending on concentration and time after treatment. While in control, the dry weight was increased after 3, 7, 15 and 25 days with 11.37, 24.76, 48.67 and 93.2% of its value at zero time, both two compounds reduced the dry weight increasing rate in comparison to control at all times and the most highest effect was obtained during the first three days after treatment at all tested concentrations. 3-Methyl-1-(2,4-dinitrophenyl)pyrazol-5-one also highly affected the dry weight during seven days after treatment.

It was more potent than 5,6-dichlorobenzotriazole nearly at all the tested concentrations exhibiting dry weight increase from -12.75 to 8.77 % and from -4.5 to 9.12% of its zero time value within the tested time after treatment at 50 μg/ml (the optimum concentration) (Figure 3). These results may indicate that 5,6-dichlorobenzotriazole acts as pre-emergent phytocidal compound, while 3-methyl-1-(2,4-dinitrophenyl)pyrazol-5-one as post-emergent phytocidal compound.

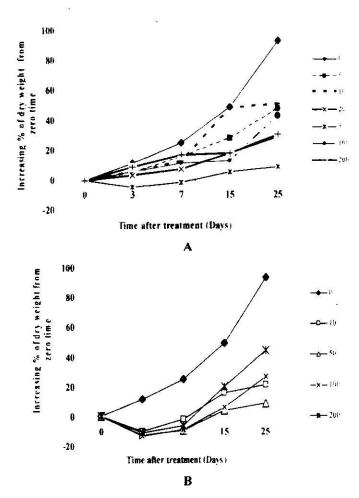


Fig. (3). Effect of post emergence treatment on wheat seedlings dry weight. A. 5.6-dichlorobenzotriazole: B, 3-methyl-1-(2,4-dinitro-phenyl)pyrazol-5-one: Concentrations in ug ml

2. Biochemical effects

2.1. Effect on soluble sugars contents

Single post-emergence treatment with both 5,6-dichlorobenzotriazole and 3-methyl-1-(2,4-dinitro-phenyl)pyrazol-5-one affected the total soluble sugars contents in a function of the tested concentration and time after treatment (Table 5, Figure 4 a & b).

Table (5). Effect of 5,6-dichlorobenzotriazole and 3-methyl-1-(2,4 dinitrophenyl)

nyrazol-5-one on wheat seedlings sugars.

| Compound | Conc. | Sugar | μg sugar/g dry plant at different days after treatment | | | | | | | |
|------------------|---------|---------|--|----------------|----------------|----------------|----------------|--|--|--|
| 5,6-Dichloro- | μg/mi | type | 0 | 3 | 7 | 15 | 25 | | | |
| | | TSS | 43.8± 2.8 | 38 5±2.5 | 35.2± 1 3 | 30.9± 1.4 | 36.5 ± 2.0 | | | |
| | 5 | RS | 28 6± 2.1 | 29 5±1 3 | 27.8± 2.5 | 23 9± 2 3 | 25.6± 2.1 | | | |
| | | Non-R S | 15.2± 1.2 | 9.0 ± 2.2 | 74±08 | 70±13 | 10.9± 2.3 | | | |
| | | TSS | 53.5± 3.4 | 37 5± 1 4 | 34 l± 1 8 | 44 ()± 2.9 | 54.2±33 | | | |
| | 10 | RS | 25.4± 2.6 | 33 9± 14 | 29 2± 1 4 | 410±27 | 37 2 ± 3 3 | | | |
| 5.6-Dichloro- | | Non-R S | 28.1± 2.1 | 3.6 ± 0.3 | 4.9± 0.4 | 3.0 ± 1.0 | 17.0± 2.8 | | | |
| henzotriazole | i i | TSS | 49 3± 2.4 | 41.9± 2.1 | 34 0± 2 7 | 28.7± 3.3 | 23.3±1.9 | | | |
| | . 1 | R.S. | 21 1 = 1 7 | 26 1: 2 3 | 25.0± 1.6 | 23.5 ± 2.3 | 14 6± 2 2 | | | |
| | | Son-R S | 28 2± 1 9 | 158:17 | 90±11 | 52:08 | 87±11 | | | |
| | | 188 | 72 2± 4 1 | 71 9± 3.5 | 37.3 ± 2.4 | 53.4± 2.7 | 82.5± 5.3 | | | |
| | 200 | R/S | 237±19 | 33 8± 1 9 | 26.5 = 2.1 | 1 21 6± 1 3 | 39 0± 3.3 | | | |
| | | Non-R S | 48.5 ± 2.1 | 383±13 | 10.8:1.7 | 31.8±1.3 | 43 5± 4 3 | | | |
| | | TSS | 554 = 53 | 10.0+3.0 | 46 1= 2 6 | 54 2± 2 3 | 61.6±4. | | | |
| | 5 | R S | 210 : 21 | 21.6±1.2 | 37.6 ± 2.8 | 32 8± 2 3 | 35 2± 2.5 | | | |
| | | Non-R S | 344±26 | 28 3±2.3 | 85±04 | 20 4± 2 3 | 26 4± 2. | | | |
| | | ESS | 734 ± 33 | 69 8= 5 1 | 47 2: 2 3 | 69.5±51 | 77.5±6. | | | |
| 3-Methvl-1-(2,4- | [0 | R S | 26.2 ± 2.3 | 474=46 | 39 8= 2 3 | 25 3r 17 | 21 6± 2 - | | | |
| dinitro-phenyl) | 2.9 | Non-R S | 47.2 ± 3.3 | 22 4 = 1 3 | 7.4± 2.3 | 44 2± 3 3 | 55 9± 3. | | | |
| pyrazol-5-one | . 81 | T.S.S | 933 - 78 | 616±43 | 46 4= 13 | 55.5±23 | 84 3± 5 1 | | | |
| | 100 | R S | 62.4 ± 5.4 | 18.5 ± 2.1 | 37.7 = 1.3 | 38 5± 2 3 | 60.5± 4 | | | |
| | 250000F | Non-R S | 30.9 ± 2.4 | 431±32 | 8.7± 0.7 | 17.0 ± 2.3 | 23.8± 1.1 | | | |
| | ļ | TSS | 795 - 53 | 60.7±3.3 | 55 7: 2 3 | - 790±46 | 798 = 6 | | | |
| | 200 | R S | 519±33 | 13.5± 1.1 | 43 9= 2 3 | 25 9 = 2.4 | 53.8± 4 | | | |
| | l | Non-R.S | 276 ± 27 | 47.2±16 | [18±11 | 53 1± 3 1 | 26.0± 2.3 | | | |

T.S.S. total soluble sugars; R.S, reduced sugars; Non-R.S. non-reduced sugars; Data are averages of three replicates

Both reduced and non-reduced sugars alternatively changed regarding the time after treatment. At the same time, total soluble sugars (TSS) in μ g/g dry plant were reduced at 5, 10 and 200 μ g/ml of 5,6-dichlorobenzotriazole up to 7 days, followed by reduction of this effect at 15 and 25 days after treatment, while its 100 μ g/ml treatment was the most effective concentration reducing TSS increasingly with time after treatment exhibiting 52.7% reduction at 25 days. Low activity at 200 μ g/ml might be referred to its difficult penetration. However 3-methyl-1-(2,4-dinitrophenyl)pyrazol-5-one showed its maximum activity after 7 days at 100 μ g/ml. Triazole structure in 5,6-dichlorobenzotriazole appeared to be more potent in reducing sugar contents than the studied pyrazole derivative, 3-methyl-1-(2,4-dinitro-phenyl)pyrazol-5-one.

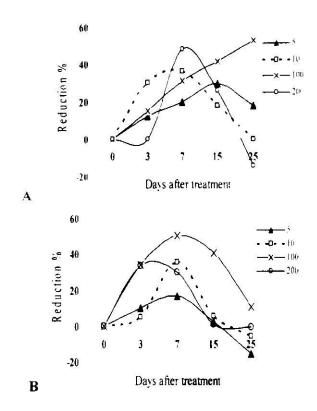


Fig. (4). Effect of post emergence treatment on wheat seedlings sugars. A. 5.6-dichlorobenzotriazole; **B.** 3-methyl-1-(2,4-dinitro-phenyl) pyrazol-5-one; Concentrations in $\mu g/ml$

2.2. Effect on chlorophyll contents

The results of post-emergence effect of 5,6-dichlorobenzotriazole and 3-methyl-1-(2.4-dinitro-phenyl)pyrazol-5-one on chlorophyll a and b contents in wheat plants are shown in Table (6). The reduction of chlorophyll contents in seedlings treated with 5,6-dichlorobenzotriazole was decreased with increasing time after treatment and the highest effect was till 3 days. At 15 days after treatment, chlorophyll contents were enhanced at all concentrations. Chlorophyll (a) was more sensitive than chlorophyll (b)(as they were reduced with 53.4 and 33.4% and with 14.0 and 17.0% at 3 and 7 days after treatment, respectively at the most effective concentration (100 µg/ml). Vice versa, 3-methyl-1-(2,4-dinitro-phenyl)pyrazol-5-one affected

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chlorophyll a less than chlorophyll b. Enhancement was noticed at low concentrations at all the tested periods. The most reducing concentration was 100 μ g/ml as it reduced chlorophyll a and b with 9.8 and 50.2 % and with 15.2 and 53.0 % at 3 and 7 days after treatment, respectively.

Table (6). Effect of post emergence treatment with 5,6-dichlorobenzotriazole and 3-methyl-1-(2,4-dinitrophenyl)pyrazol-5-one on chlorophyll in

| wheat plant | s. | | | | | | |
|--|---------|---------------|---|--|-------------------------------------|--|--|
| Compound | Conc. | Chi. | Inhibitory effect on chlorophyll at different days after treatment | | | | |
| The second secon | ⊥ μg/ml | Type | 3 | 7 | 15 | | |
| | 0 | a b | 0.0 | 0.0 0.0 | 0.0 | | |
| | 5 | a b | -20.5 ± 2.3 -46.6 ± 3.5 | -24.9 ± 2.1 -49.7 ± 3.2 | -98.6 = 2.3 -61.0 = 6.1 | | |
| | 10 | a b | 20.4 ± 2.1 -16.5 ± 1.2 | 15.7 ± 1.9 5.0 ± 3.1 | -10.1 ± 3.4 -11.2 ± 2.1 | | |
| 5.6-Dichlorobenzotriazole | 20 | a b | 24.6 ± 3.0 -15.2 \pm 2.6 | 14.3 ± 2.8 -21.8 ± 1.2 | -27.0 ± 3.1 -35.6 ± 6.2 | | |
| | 50 | a b | 31.8 ±3.5 1.8 ± 0.8 | 30.5 ± 5.3 12.0 ± 2.4 | -28.5 ± 2.3 -19.5 ± 3.5 | | |
| | 100 | a b | 53.4 ±6.2 14.0 ±2.5 | 33.2 ± 6.2 17.3 ± 2.1 | -45.5 ± 6.4 -33.5 ± 6.3 | | |
| | 200 | a b | 24.9 ±3.1 1.2 ±0.9 | 15.3 ± 2.4 12.7 ± 3.1 | -38.4 ± 3.2 -18.5 ± 2.1 | | |
| | 0 | a b | 0.0 0.0 | 0.0 | 0.0 | | |
| | 5 | a b | -18.6 ± 2.6 -10.8 ± 2.4 | -34.6 ± 1.2 -17.1± 3.2 | -87.2 ± 5.7 -81.0 ± 5.6 | | |
| | 10 | a b | - 25.7 ± 3.1 -1.3 ± 10 | -41.7 ± 1.2 35.2 ± 3.6 | - 135.9 ± 12.3 | | |
| 3-Methyl-1-(2,4-dinitro- | 20 | a b | -54.2 ± 3.4 -29.2 ± 3.6 | -62.4 ± 2.8 -18.7 ± 2.4 | -135.4 ± 8.7 -20.5 ± 2.1 | | |
| phenyl)pyrazol-5-one | 50 | a b | 9.2 ± 2.5 10.1 ± 3.4 | 29.5 ± 6.2 30.7 ± 5.4 | -14.6 ± 3.1 -98.2 ± 6.3 | | |
| | 100 | a b | 9.8 ± 3.6 15.2 ± 2.7 | 50.7 ± 3.4 50.2 ± 6.1 53.0 ± 3.9 | -38.7 ± 3.7 -26.8 ± 6.2 | | |
| | 200 | a b | -45.2 ± 9.4 -23.5 ± 6.1 | -28.7 ± 2.9 27.6 ± 4.5 | -107.6 ± 8.7 -22.6 ± 3.2 | | |

(-) stimulation; a, chlorophyll a; b, chlorophyll b

2.3. Effect on soluble phenolics

Soluble phenolics are calculated in mg gallic acid equivalent/g fresh seedling and recorded in Table (7). The results indicated that treatment with 5.6-dichlorobenzotriazole reduced the soluble phenolics content mostly until 3 days after treatment at all concentrations systematically with increasing the tested concentration. This effect was fluctuated according to the tested concentration at 7 days after treatment. The most effective concentration was 100 µg/ml. At 15 days after treatment, it was too long to keep its effectiveness in reducing soluble phenolics content. While 3-methyl-1-(2,4dinitrophenyl) pyrazol-5-one caused reduction of their content at all the tested concentrations in non-systematic arrangement. This effect was continued up to 15 days after treatment. These effects on chlorophyll content disturb several physiological processes in plants. The effect on soluble phenolics interfere in the protective compounds. Ferreres et al. (2009) referred the protective effects of barely leaves to their contents of flavones and soluble phenolics. At the same time, Ruhland et al. (2007) revealed the positive relation between the ferric reducing antioxidant power (FRAP) and the bulk-soluble phenolic concentration.

Table (7). Effect of 5,6-dichlorobenzotriazole and 3-methyl-1-(2,4-dinitrophenyl)pyrazol-5-one on soluble phenolics in wheat

| plants | <u>s. </u> | | | 1500 | | | | | |
|--|---|--|---------------|---------------|---------------|--------------|---------------|---------------|--|
| Compound | Days | Effect on soluble phenolics after different concentration: (µg/ml) | | | | | | | |
| 5.6- Dichlorobenzotriazole | | 0 | 5 | 10 | 20 | 50 | 100 | 200 | |
| 5,6- Dichlorobenzotriazole | 3 | 7.9 ± 0.39 | 6.9 ± 0.13 | 5.9 ± 0.77 | 5.8 ± 0.10 | 5.6± 0.26 | 5.4 ± 0.29 | 5.6 ± 0.03 | |
| | 7 | 5.3 ± 0.01 | 5.2 ± 0.23 | 6.3 ± 0.32 | 8.1 ± 0.39 | 3.8 ± 0.32 | 9.3 ± 0.81 | 8.3 ± 0.03 | |
| | 15 | 4.4 ± 0.32 | 5.8 ± 1.68 | 5.9 ± 2.88 | 6.3 ± 0.20 | 7.1 ± 2.26 | 12.5 ± 0.16 | 11=1 | |
| | 3 | 6.7± 1.10 | 6.8± 1.26 | 5.1± 0.81 | 4.4± 1.00 | 4.3± 1.42 | 6.6± 2.82 | 9.0± 4.68 | |
| 3-Methyl-1-(2,4- dinitro phenyl) pyrazol-5-one | 7 | 12.0± 0.29 | 8.1± 3.20 | 7.7± 1.65 | 6.7± 1.20 | 6.3± 2.2 | 5.6± 0.62 | 9.4± 0.68 | |
| F.: | 15 | 10.5± 0.81 | 7.3± 3.65 | 8.4± 0.42 | 6.4± 0.42 | 5.8± 2.26 | 7.6± 1.36 | 8.5± 0.74 | |

Data are mg gallic acid equivalent ± SD/gm fresh weight

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The fluctuated results of chlorophyll and soluble phenolics may be due to the interactive effects of temperature and the accumulated soluble phenolics of gallic acid equivalent per 100 g of wheat seedlings (Oncel et al., 2000). They proved the interactive effects of temperature and heavy metal stress on wheat seedlings growth through changes in length, percentage of dry weight, chlorophyll (a, b) as well as total soluble phenolics and biochemical effects of the active derivatives.

It could be concluded that pre-emergence treatment of wheat with 5,6-dichlorobenzotriazole inhibited seed germination and seedling growth in addition to high inhibition of root and shoot systems growth in wheat (T. aestivum) and squash (C. pepo) seedlings more than the used standard herbicide (metribuzin) in this respect. It reduced fresh and dry weights of wheat seedlings. 5,6-Dichlorobenzotriazole was more effective to decrease the dry weight of wheat seedlings in the pre-emergence more than in the post-emergence treatment. Vice versa, in case of 3-methyl-1(2,4-dinitrophenyl)pyrazol-5-one.

These results may also prove that 5,6-dichlorobenzotriazole does not affect photosynthesis through only inhibiting the chlorophyll content going with Luo et al. (2008) as they revealed that triazole derivatives inhibit cell division and protoporphyrinogen oxidase leading to membrane disruption and inhibiting photosynthesis. Its also affected by increasing cellular peroxides, hexose phosphates, oxidized glutathione and glucose-6-phosphate dehydrogenase activity as revealed in treatment of wheat (T. aestivum L.) leaves with 3-Amino-1,2,4-triazole, which is a specific inhibitor of catalase (Okuda et al., 1992).

Benzotriazole derivatives are effective in blocking photosynthetic circumster (Sung et al., 1991) and their most important influence is to slow down the growth and decrease the plant size emphasizing the obtained results on fresh and dry weight. (Beek, 1987). They inhibit protein kinases CKI and CKII (Szyszka et al., 1995). They also affect through over production of reactive oxygen as H₂O₂, which its accumulation is generally considered to contribute to oxidative damage and probably process signaling. Among the studied benzotriazole derivatives, 5,6-dichlorobenzotriazole was the strongest derivative because of presence of 5,6-dichloromoiety as electron withdrawing groups, which are the most desirable for high activity (Sung et al., 1991).

The effects of the studied indole derivatives were also varied based on their structure differences and their effects may be due to the used concentrations as increasing concentrations of IAA, and Auxin herbicides induced growth abnormalities leading to desiccation, tissue necrosis, and decay. They also increased levels of H₂O₂, which contributes to the induction of cell death, deoxyribonuclease (DNase) activity and chlorophyll loss as sensitive indicators for the progression of tissue damage (Grossman, 2003). Pyrazole derivatives are considered as branched chain amino acid synthesis (ALS or AHAS) inhibitor. They act by inhibiting biosynthesis of the essential amino acids valine and isoleucine, hence stopping cell division and plant growth.

REFERENCES

- Abdel-Aty, A. S. (1996). Rodenticidal activity of certain organic molecules "Chemistry and rodenticidal activity of some benzotriazole and coumarin derivatives". MSc, Pesticide Chem. Department, Faculty of Agriculture, Alexandria University, Egypt.
- Abdel-Aty, A. S. (2007). Pesticidal activities of some pyrazole derivatives. J. Adv. Agric. Res., 12 (4): 783-793.
- Abdel-Aty, A. S. (2010). Fungicidal activity of certain indole derivatives against some plant pathogenic fungi. J. Pestic. Sci., 35 (4), 431–440.
- Abdel-Aty, A. S. and Mohareb, A. S. O. (2008). Preliminary evaluation of certain benzylidine and pyrazole derivatives against wood decay fungi. J. Pest Cont. Environ. Sci., 16 (1/2): 111-125.
- Ahmed, S. M.; Abdel-Aty, A. S. and Desheesh, M. A. (2004). Fungicidal effects of certain benzotriazole and coumarin derivatives. Alex. Sci. Exch., 25 (2): 321-330.
- Beek, J. R. (1987). Plant Growth Regulating Triazoles [P]. EP 0227284 A1, 1987-01-07.
- Caramazza, R.; Cereti Mazza, M. T. and De Cicco, L. (1990). Preparation and activity of metal complexes with heteroatomic organic ligands. Preliminary note. Boll Soc Ital Biol Sper., 66 (8):717-24.

- Cohort Software Inc. (1986). Costat user's manual, version 3.03. Berkeley. California, USA.
- Ferreres, F.; Krsková, Z., Gonçalves, R. F.; Valentão, P.; Pereira, J. A.; Dusek, J.; Martin, J. and Andrade, P. B. (2009). Free water-soluble phenolics profiling in barley (*Hordeum vulgare* L.). J. Agric. Food Chem., 57 (6): 2405-2409.
- Grodzinsky, A. M. and Grodzinsky, D. M. (1973). Short reference in plant physiology. Naukova Domka, Rev, R. U.S.: 433-34.
- Grossmann, K. (2003). Mediation of herbicide effects by hormone interactions J. Pl. Grow. Regulat., 2: 109-122.
- Hipkins, M. F. and Baker, N. R. (1986). Photosynthesis energy transduction. Spectroscopy, IRL Press, Oxford, Washington: 51-101.
- Horii, A.; McCue, P. and Shetty, K. (2007). Seed vigour studies in corn, soybean and tomato in response to fish protein hydrolysates and consequences on phenolic-linked responses. Bioresource Technol., 98: 2170-2177.
- Kumar, V.; Kumar, A. and Kharwar, R. N. (2007). Antagonistic potential of fluorescent pseudomonads and control of charcoal rot of chickpea caused by *Macrophomina phaseolina*. J. Environ Biol., 28 (1): 15-20.
- Luo, Y.P.; Jiang, L. L.; Wang, G. D.; Chen, Q. and Yang, G. F. (2008). Synthesis and herbicidal activities of novel triazolinone derivatives. J. Agric. Food. Chem., 56 (6):2118-24.
- McCue, P.; Zhheng, Z.; Pinkham, J. L. and Shetty, K. (2000). A model for enhanced pea seedling vigour following low pH and salycilic acid treatments. Process Biochem., 35: 603-613.
- Okuda, T.; Matsuda, Y.; Sugawara, M. and Sagisaka, S. (1992). Metabolic response to treatment with cold, paraquat, or 3-amino-1,2,4-triazole in leaves of winter wheat. Biosci Biotechnol Biochem., 56 (12): 1911-1915.

- J. Pest Cont. & Environ. Sci. 19, NO. 1, 15-37 (2011)
- Oncel, I.; Keleş, Y. and Ustün, A. S. (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environ. Pollut., 107 (3): 315-320.
- Quinn, J. A.; Fujimota, T. T.; Egan, A.R. and Shaber, S.H. (1985). The properties of RH 3866, a new triazole fungicide. Pestici. Sci., 17 (4): 357-362.
- Robin, G. (1986). Induction of male sterility in wheat, *Triticum aestivum* using organic ligands with high specificity for binding copper. Euphytica, 35 (2): 621-630. (c.f. B.A., 82 (10): 88703, 1982).
- Ruhland, C.T.; Fogal, M. J.; Buyarski, C. R. and Krna, M. A. (2007). Solar ultraviolet-B radiation increases phenolic content and ferric reducing antioxidant power in *Avena sativa*. Molecules 12 (6): 1220-1232.
- Ryu, C. K.; Lee, J. Y.; Park, R. E.; Ma, M.Y. and Nho, J. H. (2007). Synthesis and antifungal activity of 1H-indole-4,7-diones. Bioorg Med Chem Lett., 17 (1): 127-131.
- Sharaf, E. F. and Farrag, A. A. (2004). Induced resistance in tomato plants by IAA against *Fusarium oxysporum lycopersici*. Pol. J. Microbiol., 53 (2): 111-6.
- Staab H. A. and Rohr, W. (1968). In Newer Methods of Preparative Organic Chemistry (W. Foerst, ed.) vol. 5, pp. 61 -108, Academic Press. New York.
- Sung, N. D.; Park, H. J; Park, S. H. and Pyon, J. Y. (1991). Herbicidal activity and molecular design of benzotriazole derivatives. J. Korean Agric. Chem. Soc., 34 (3): 287-294.
- Szyszka, R.; Grankowski, N.; Felczak, K. and Shugar, D. (1995). Halogenated benzimidazol-es and bezotriazoles as selective inhibitors of protein kinases CKI and CKII from *Saccharomyces cerevisiae* and other sources. Biochem. Biophys. Res. Comm., 208 (1): 418-424.

- Thomas, W. and Dutcher, R. A. (1924). Picric acid method for Carbohydrate, J. Am. Chem. Soc., 46: 1662-1669.
- Watanabe, N.; Horikoshi, S.; Kawasaki, A.; Hidaka, H. and Serpone, N. (2005). Formation of refractory ring-expanded triazine intermediates during the photocatalyzed mineralization of the endocrine disruptor amitrole and related triazole derivatives at UV-irradiated TiO₂/H₂O interfaces. Environ. Sci. Technol., 39 (7): 2320-2326.
- Yasuor, H.: TenBrook, P. L.; Tjeerdema, R. S. and Fischer, A. J. (2008). Responses to clomazone and 5-ketoclomazone by *Echinochloa phyllopogon* resistant to multiple herbicides in Californian rice fields. Pest Manag Sci., 64 (10):1031-1039.
- Zemanek, J. (1963). The method of testing the effectiveness of herbicides on agar medium Rostle. Vyroba, 9: 621-632 (c.f. Weed Abstracts, 2 No 1130, 1963).

النشاط الإبادي لبعض مشتقات الأزول على النباتات

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تم تقييم مجموعة من مشتقات الأزول من حيث سميتها النباتية على إنبات البذور و كذلك نمو كل من المجموع الخضرى و الجذرى على نبات القمح كوحيد الفلقة (رفيعة الأوراق) والكوسة كثنائى الفلقة (عريضة الأوراق). خلال هذه الدراسة تم تقييم سنة من مشتقات الإندول (تحتوى ذرة نيتروجين واحدة) و كذلك أربعة من مشتقات البيرازول (تحتوى ذرتى نيتروجين) بالإضافة إلى سنة مشتقات لمركبات البنزوثلاثى الأزول (تحتوى ثلاث ذرات نيتروجين) السابق تحضيرها معمليا.

أوضحت النتائج أن التائير الإبادي ظهر كدالة في التركيب الكيماوي و التركيز للمشتق كما أظهرت أن كل المركبات المختبرة كانت أكثر تأثيرا على نمو النبئة منها على عملية الإنبات نفسها و ذلك عنما طبقت قبل الإنبات محدثة بعض صور التقزم و التشوه في جذور البادرات النامية.

من خلال التقييم المبدنى بالتطبيق قبل الإنبات و كذلك باجراء تجارب الأجار المانى وجد أن مركب الدى 6- ثنانى كلورو بنزوثلاثى الأزول (3) ظهر أعلى هذه المركبات سمية مثبطا عملية الإنبات و كذلك نمو البادرات النامية بتركيز مثبط لـ 50% من العشيرة المدروسة قدره 10.5 و 5.0 ميكروجرام/ مل على التوالى متعديا بذلك مبيد الحشائش المستخدم للقياس ميتروبيوزين (سنكوز). أخمد نفس المركب نمو المجموع الجذرى و المجموع الخضرى لنبات الكوسة (ذات الغلقتين) بتركيز مثبط لـ 50% قدره 8.6 و 16.8 ميكروجرام/ مل على التوالى , في حين ظهر أكثر سمية بتركيز مثبط لـ 50% قدره 3.6 و 12.4 ميكروجرام/ مل على التوالى في حالة نبات القمح (أحادى الفلقة).

طهر مركب الـ 3- ميثايل-1- (2 ,4- ثنانى نيتروفينيل بيرازول-5- ون) (17) أعلى مشتقات البيرازول سمية خافضا نمو المجموع الجذرى و الخضرى بـ 47.2 و 77.1 ميكروجرام/ مل على التوالى على النبات عريض الأوراق المستخدم.

رش المركبين الأكثر فعالية (3 ، 17) مرة واحدة بعد الإنبات لوحظ أنها قد سببت تأثيرا حادا إلى معتدلا على معتويات النبات المعامل من الكلوروفيل (أ, ب). محتوى الفينولات الذائبة و ظهر أفضل تأثير على تركيز 100 ميكروجرام/ مل. تأثر أيضا محتوى البادرات من السكريات المختزلة و غير المختزلة بالإضافة إلى الوزن الجاف المنبات بنسبة إختزال وصلت 94.2 %. ظهر هذا التأثير بقوة إختلفت بإختلاف المركب المطبق. التثير بقوة إختلف المركب المطبق. يستخلص من هذا أن مركب الـ5, 6 ثنائي كلورو بنزوثلاثي الأزول هو أفضل المركبات المستخدمة تأثيرا على نمية الملانبات و كذلك نمو البادرات المعاملة . كما أحدث خللا داخل النظام الحيوى النبات معتمدا على التركيز و الزمن بعد المعاملة .