P 15. ESTIMATION OF MANCOZEB-BASED FUNGICIDES AS POTENTIAL POLLUTANTS BY INDUCEMENT OF PHYTO- AND GENOTOXICITY ON ALLIUM CEPA L. ALBANIAN ECOTYPE DRISHTI

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ABSTRACT: Pesticide pollution issues are increasingly occurring all over the world. In order to estimate the potential polluting effects of some mancozeb-based fungicides the present study investigated their phytotoxicity and genotoxicity on seeds and root meristematic cells of *Allium cepa* L. Albanian ecotype Drishti. Seed germination capacity, root length, evaluation of EC_{50}-s, mitotic index, frequencies of micronuclei, chromosomal aberrations and types were applied as toxicity indicator parameters after the treatment under three time exposure periods (24, 36 and 48 hours) of biological materials with four concentrations (0.04-0.16%) of AGRIA-MANCOZEB 80 WP, MANFIL 75 WG and DITHANE M-45 blue 72 WP. The result revealed limitation in seed germination and significant root growth restriction mainly after 36- and 48 hours exposure of Manfil and AGRIA-MANCOZEB, having even EC_{50} values included into field applications. Obvious reduction of meristematic activity and increased chromosomal abnormalities and micronuclei incidence were recorded particularly at the highest concentrations of fungicide samples after 48 h exposure. The current data distinctly emphasized the phyto- and genotoxic effects on a non-target crop and assay as onion of all investigated mancozeb-based fungicides, broadly used in Albanian agriculture during the last decade, demonstrating their potential pollution impact on environment and human population health. Our findings should serve as a prominent alert for the prospective risk situation caused from indiscriminate use of fungicides, their active ingredient purity and toxic consequences on food chain organisms.

Keywords: Environmental pollution, Mancozeb-based fungicides, Allium cepa L. assay

1. INTRODUCTION

Extent and persistent use of a broad pesticides spectrum has created the problematic occurrence of environmental pollution issues all over the world. Despite the enormous economical profits of farmers, only a small part of applied pesticide doses achieves the targeted pest (USEPA, 2005) consequently impairing each environmental component and respective biota. Missmenagement and even abuse of fungicides application in particular directly engender to degradation of soil fertility and to the quantity and quality of crops production. The contaminated crop yield due to accumulation of fungicide residues is getting a serious threat for the health of livestock and human consumers. The data of scientific reports during the last decades are plausible to motivate further assessment of potential toxic effects of certain fungicide groups on non-target agricultural plants at biochemical, cytogenetic and physiological level. According to Gupta (2018) more than 80% of all oncogenic risk from the use of pesticides derives from a few fungicides.

Mancozeb-based fungicides appertain to the ethylenebisdithiocarbamates cathegory of synthetically produced pesticides. They are labelled to control acting by contact on fungi development and dispersion through different crops, fruit trees, forestry, decorative plants, garden and park lawns and hedges. Mancozeb itself consist in a mixture of [1,2ethanediylbis] carbamodithioate](2-)]manganese with [1,2-ethanediylbis [carbamodithioate](2-)zinc salt. More than $1/3^{rd}$ of total sold mancozeb as active ingredient is usually applied in co-formulation with other fungicide classes or pesticides (Runcle et al., 2017). Derivatives of mancozeb as ethylene bisisothiocyanate sulfide and ethylene bisisothiocyanate directly harm the mitochondrial and cytoplasmatic activity of sulphydryl group containing enzymes, thus inhibiting important biochemical processes into fungal cells (Gullino et al., 2010). Mancozeb-based fungicides are present in plant protection programs from pests and pesticide markets by more than 70 years, but periodic scientific reports are often contradictory according to the ecotoxicological

consequences. Last decades studies evidence the deleterious effects on water quality, soil enzymes, different species of soil bacteria, plants, nematodes, aquatic invertebrates, fishes, mice, rats and even human health (Houeto et al., 1995; Steenland et al., 1997; Easton et al., 2001; Untiedt and Blanke, 2004; USEPA, 2005; Corsini et al., 2006; Mubeen et al., 2006; Rossi et al., 2006; Cecconi et al., 207; Axelstad et al. 2011; Tripathi et al., 2011; Dias et al., 2014; Atamaniuk et al., 2014; Walia et al., 2014; Pavlic et al., 2015; Hoffman et al., 2015; López-Fernández et al., 2016; Saha et al. 2016; Todt et al. 2016; Runkle et al. 2017; Kwon et al. 2018; Morales-Ovalles et al., 2018; Palmerini et al., 2018; Yahia e t al., 2019).
The benefit of toxicological databases co-structured on bio-monitoring information is to establish the adequate biological models for the evaluation and prediction of potential effects of chemicals, being thus an important objective of public health, with the intention to avoiding or minimize direct and indirect living beings and human exposure to toxic and even mutagenic substances (Rouabhi, 2010; Elskus, 2012; USEPA, 2018).. They can give comprehensive data according to additive, synergic or antagonist bio-effects at organism (toxicity) and molecular (genotoxicity) levels, which may not be easily identified with specific physic-chemical analyses (Power and Boumphrey, 2004). The use of higher plants as biotests has been standardized and is widely utilized, because they present several advantages compared with other organisms such as: high reproductive capacity, short-term and easy application during *in vivo, in vitro* and *in situ* methods, more ethically appropriate compared to animal tests and low cost (Kristen, 1997; Grant, 1999; Maluszynska and Juchimiuk, 2005; Ma et al., 2005; Mesi et al., 2012; Iqbal et al., 2019). Being a biological early warning tool in short-term monitoring procedures, the *Allium cepa* L. (2n=16) test is broadly applied for the detection at morpho-, cyto- and genetic level of toxic effects induced from contaminant substances (Fiskesjö, 1993; 1994; 1997; Leme & Marin-Morales, 2009; Tedesco and Laughinghouse, 2012; Khannah and Sonia, 2013; Bonciu et al., 2018), pesticides in general (Antonise-Wiez, D. 1990; Nithyameenakshi et al., 2006, Feretti et al., 2007; Asita and Matebesi, 2010; Turkoğlu, 2012; Mesi and Kopliku, 2013; 2015; Pratte-Santos 2015; Boumaza et al., 2016; Dizdari et al., 2017; Dizdari and Bala, 2019) and mancozeb-based fungicides in particular (Barakat et al., 2010; Fatma et al., 2018).
The present study is focused on the estimation of three mancozeb-based fungicides (commonly used in Albanian agriculture) AGRIA-MANCOZEB 80 WP, MANFIL 75 WG and DITHANE M-45 blue 72 WP as potential pollutants by assessing the phyto- and genotoxicity effects on *Allium cepa* L. native ecotype Drishti assay.

2. MATERIAL AND METHOD

2.1. Treatment solutions

The mancozeb-based fungicides AGRIA-MANCOZEB 80 WP, MANFIL 75 WG and DITHANE M-45 blue 72 WP were purchased from the Albanian pesticide markets. Drinking water was used as negative control (NC) and to dilute the fungicide samples obtaining four definitive concentrations: 0.04, 0.08, 0.12 and 0.16% applied to establish the further full-scale toxicity test.

2.2. Biological material and test procedures

Healthy-looking and uniform size seeds and bulbs of *Allium cepa* L. native ecotype Drishti were used as biological material and kept at room temperature (22±0.2° C). The germinating seeds and newly emerged roots of onion bulbs were exposed for 24, 36 and 48 hours to the chosen concentrations of mancozeb-based fungicides. Sets of 20 seeds per sample were formerly sterilized with NaOCl 50%, soaked for 24 h in distilled water and then allowed to germinate in 18.5 cm Petri dishes between two layers of sterile moist cotton with a total of 28 respective treatment solutions. Seeds were considered fully germinated, if their radicle was apparently emerged. Simultaneous experiments were set up with ten test tubes per fungicide, concentration and exposure period sample, overturning the bulbs with the root primordia into the liquid, after removing the outer dry scales. An additional recovery period till 96 hours with drinking water (NC) followed the treatment of both biological materials after respective exposure times with different fungicide and concentrations to permit the evaluation of mean root length (MRL) and EC_{50} values of onion bulbs and seed germination capacity as phytotoxity parameters. Seed germination capacity (GC) was expressed as a percentage of germinated seeds to the total number of seeds per champion. The corresponding EC_{50} values (effective concentrations of different chemicals and mixtures, permitting 50 % growth of the sample under

study in relation to control) of assessed Mancozeb-based fungicides were statistically evaluated by plotting on graph root length values as percentage to negative control against treatment concentrations and using the trend-line equations with the biggest R^2 values (polynomial, order 3. To examine cytotoxic and genotoxic effects of tested fungicides and even damage fixation on meristematic cells the following microscopic endpoints: mitotic index (MI), frequencies of aberrant mitotic cells (FAC), chromosomal aberrations (CA) types and interphase cells with micronuclei (FMN) in root meristem, were observed and quantified. Root tips of 10 mm taken from 5 bulbs, randomly chosen in each sample series, were placed on slides and the terminal root tips (1-2 mm) were cut off and used for further preparation of microscopy slides, in accordance with the standard procedure for orceine staining of squashed material (Singh, 2016). The total number of dividing cells (NDC) was determined in 1000 examined cells in the field of view per each slide, then MI was scored as percent ratio of NDC. The formation of micronuclei was examined in about 1000 cells per slide at interphase, taking in account only the cells with intact cellular and nuclear membranes. 1500 dividing cells (300 cells/slide) have been observed for the characterization and classification of chromosome aberrations (CA). The frequencies of micronuclei (MNC), aberrant cells (FAC) and CA types were expressed as percent ratio.

2.3 Statistical analyzes

Analysis of Variance One-way ANOVA and post-hoc Student Newman-Keuls (SNK) tests were used to test for significant differences of all evaluated parameters in *A. cepa* L. seeds and roots, exposed to different mancozed-based fungicides, concentrations and exposure times. All the results were expressed as the mean of three replicates per sample. Parameter differences between fungicide treatments, duration and corresponding NC-s were considered statistically significant at level 5%.

3. RESEARCH FINDINGS

The data related to the morphological and cyto-genetic analyzes made in the current investigation on *A. cepa* L. native ecotype Drishti seeds and rootlets are represented in tables 1 and 2 figures 1-3. It can be punctuated that the estimated parameter values differed mostly in dependent manner from tested mancozeb-based fungicides, concentration treatments and exposure time, displaying successive phyto- and genotoxicity induced (with lot significant changes as compared to the respective NC values, second ANOVA and SNK tests).

Table 1. Phytotoxic effects of Mancozeb-based fungicides on seeds and roots of *A. cepa* L.

Exposure time (h)	Tested solutions	Conc. (%)	GC±SD (%)	MRL±SD (cm)	MI±SD (%)
	NC	0	91.7±5.3	5.56±0.76	15.68±1.62
24	AGRIA-MANCOZEB 80 WP	0.04	84.6±6.0	4.95±0.69^{a}	14.43±0.96
		0.08	86.4±3.3	4.78±0.55 ^{a}	13.79±1.05^{ab}
		0.12	80.1±5.7a	4.61±0.88^{a}	12.70±0.89^{a}
		0.16	74.7±4.9ab	4.39±0.43^{b}	11.93±0.44^{b}
	MANFIL 75 WG	0.04	87.3±6.2a	5.06±0.51^{a}	14.74±1.06^{a}
		0.08	80.1±4.5ab	4.72±0.38^{a}	12.86±1.01ab
		0.12	74.6±2.8b	4.55±0.29^{a}	12.23±0.66*b^{c}
		0.16	69.1±3.9b	4.23±0.16*^{b}	11.45±0.90*bc
	DITHANE M-45 blue 72 WP	0.04	112.8±8.4	5.45±0.82	14.58±1.33a
		0.08	83.7±4.1a	5.22±0.32	13.64±1.12^{ab}
		0.12	77.3±2.9ab	4.89±0.27^{a}	12.86±0.75ab^{b}
		0.16	72.8±3.1b	4.65±0.54^{a}	12.17±0.98*bc
36	AGRIA-MANCOZEB 80 WP	0.04	77.2±5.6ab	4.47±0.46^{ab}	11.76±0.69*^{bc}
		0.08	72.6±3.5b	3.61±0.53*^{c}	10.82±1.06**^{c}
		0.12	67.3±4.9*bc	3.34±0.29**^{e}	8.14±0.53**f
		0.16	62.8±3.8*b	3.01±0.66**^{h}	7.63±0.14**
	MANFIL 75 WG	0.04	71.0±5.8b	3.89±0.41*^{bc}	10.66±0.74**^{cd}
		0.08	69.2±2.7b	3.56±0.39*^{cd}	9.41±0.38**^{e}
		0.12	64.5±3.0*c	3.17±0.22**^{eh}	9.25±0.78**^{e}
		0.16	57.3±1.9*d	2.67±0.17**^{h}	8.62±0.14**^{hi}

	DITHANE M-45 blue 72 WP	0.04	79.2±6.2ab	4.17±0.55*^{ab}	12.39±0.66*^{b}
		0.08	70.1±4.1c	3.94±0.28*^{b}	11.29±0.81*^{c}
		0.12	66.4±2.5c	3.67±0.49*^{cd}	10.07±0.49**^{de}
		0.16	61.9±4.0*cd	3.39±0.46*^{de}	9.26±0.57**^{e}
48	AGRIA-MANCOZEB 80 WP	0.04	69.1±3.3bc	3.74±0.38*^{c}	9.59±0.45**^{e}
		0.08	65.5±1.9c	3.52±0.44*^{d}	9.08±0.62**^{ef}
		0.12	59.2±4.7*d	3.13±0.10**^{e}	7.93±0.21**^{f}
		0.16	53.7±2.2*de	2.45±0.32**^{hi}	7.01±0.07**^{g}
	MANFIL 75 WG	0.04	64.6±4.4*bc	3.59±0.58*^{cd}	8.93±0.73**^{ef}
		0.08	60.1±2.7*d	3.22±0.10**^{e}	7.52±0.39**^{g}
		0.12	54.4±0.9*e	2.96±0.17**^{h}	6.87±0.28**^{gi}
		0.16	50.2±1.2*f	2.17±0.09**^{i}	6.11±0.19**^{i}
	DITHANE M-45 blue 72 WP	0.04	71.8±5.6bc	4.08±0.79*^{bc}	10.35±0.65**^{d}
		0.08	62.7±4.6*cd	3.61±0.35*^{cd}	9.72±0.33**^{e}
		0.12	58.2±3.1*d	3.31±0.26**^{de}	8.60±0.47**ef
		0.16	54.9±1.6*	2.61±0.12**^{h}	7.88±0.25**^{f}

Means labelled with asterisks and letters within columns are significantly different from respective NC-s according to One-Way ANOVA test (* P<0.05; ** P<0.001) and between exposure periods and fungicide concentrations in SNK test (p<0.05). NC-negative control; MRL-mean root length; MI-mitotic index; SD – standard deviation.

The results (Tab. 1 and Fig. 1) showed that AGRIA-MANCOZEB 80 WP, MANFIL 75 WG and DITHANE M-45 blue 72 WP application induced limitation of germination capacity of *A. cepa* seeds, particularly at the longest exposure and highest concentration treatments.

Significant reduction started at 0.12% concentration (after 36 h) under MANFIL 75 WG solution, where GC decreased with 29% of corresponding NC value (91.7%, P<0.05) The same fungicide formulation predominantly decreased GC through concentrations and time exposures raising the minimal value at 0.16% cc after 48 h (57.5%, as compared with the GC under 0.04% treatment after 24 h, p<0.05 using SNK test).

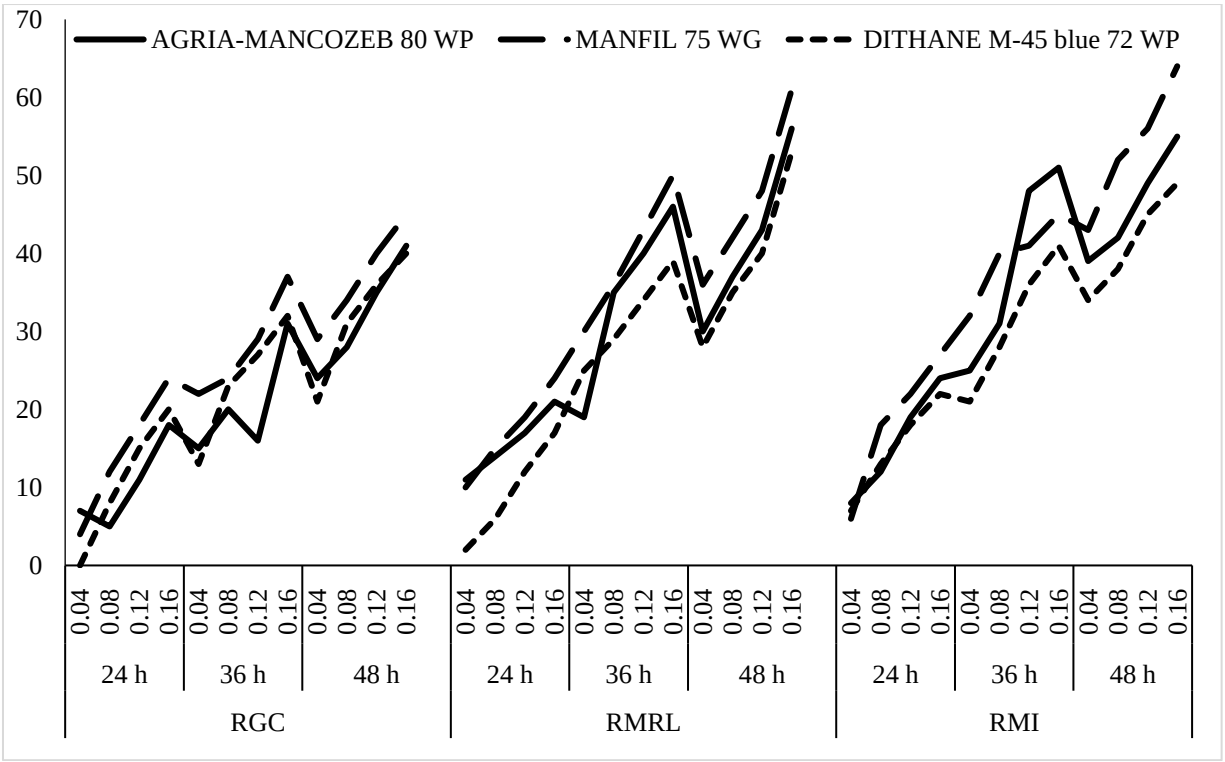

Figure 1. Comparative phytotoxicity induced on *A. cepa* L. assay by selected mancozeb-based fungicides (respective cc-s in %). RGC, RMRL, RMI – reductıons of germınatıon capacıty, mean root length and mitotic index (expressed in % of respective NC values)

The longitudinal growth (MRL) and the mitotic activity (MI) of emerging rootlets from onion bulbs were respectively reduced (as compared to the respective values of control) at: 11-56% and 8-55% by AGRIA-MANCOZEB 80 WP; 10-61% and 6-64% by MANFIL 75 WG; 2-53% and 7-49% by DITHANE M-45 blue 72 WP after bulbs exposure for 24-48 hours at concentrations 0.04-0.16% of each fungicide (Fig. 1 and Tab. 1). Significant differences of MRL and MI from corresponding NC-s (5.56 cm and 15.68 %, $P<0.05$) were firstly observed after the shortest exposure (24 h) at the highest concentration 0.16% of MANFIL 75 WG (for MRL) and 0.12% (for MI), while the first cumulative toxic effects on MRL (over 55% of corresponding NC) were found out only on bulbs treated with 0.16% cc-s of AGRIA-MANCOZEB 80 WP and MANFIL 75 WG after 48 h. Additionally, AGRIA-MANCOZEB 80 WP induced the first sublethal effect on proliferation activity of root meristem after 36h exposure under the most concentrated treatment, while no lethal effect on mitosis was observed. A sloping significant reduction ($p<0.05$) of both MRL and MI from the lowest cc-s of the shortest time exposure to the highest and longest ones resulted across all tested fungicides, particularly under MANFIL treatment where after 48h in 0.16% concentration MRL and MI resulted only 43 and 41% of respective values screened under 0.04% concentration after 24 h exposure. The extrapolated EC_{50} values used as a phytotoxicity threshold endpoint in the current study indicated that AGRIA-MANCOZEB 80 WP, MANFIL 75 WG and DITHANE M-45 blue 72 WP dimidiated the longitudinal root growth at the respective concentrations: 0.147%, 0.128% and 0.155% (Fig. 2).

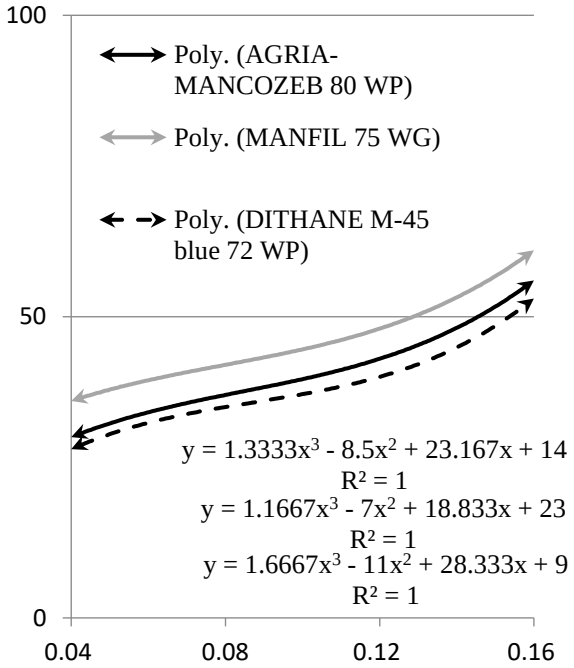

Figure 2. Evaluation of the EC_{50} –s of selected mancozeb-based fungicides (respective cc-s in %)

The data of undertaken microscopy investigation detected a considerable presence of chromosomal aberrations, positively correlated with the addition of tested fungicides' concentrations and exposure duration (Tab. 2 and Fig. 3). Recorded FAC values resulted significantly higher since at the shortest time treatment of onion roots (24h) by 0.08% concentration of AGRIA-MANCOZEB 80 WP and MANFIL 75 WG, exceeding by 253 and 281% the NC value of 1.17% ($P<0.05$). The most increasing slope of the chromosomal aberrations incidence was induced by 36h concentration treatments resulting respectively: 3-8 (AGRIA-MANCOZEB 80 WP); 4-11.5 (MANFIL 75 WG) and 2.7-6.9 (DITHANE M-45 blue 72 WP) folds higher than NC ($P<0.05$ and $P<0.01$, such was the case of all MANFIL cc-s). It could be noticed only a slightly greater significance of 48 h FAC values between the same concentrations as compared to 36h ones for MANFIL and DITHANE fungicides ($p<0.05$), contrariwise from AGRIA-MANCOZEB, where 0.16% concentration multiplied 4.7 folds the observed quantity of meristematic aberrant cells having CA with individualized ones under 24 h 0.4% cc. The types of

chromosomal aberrations recorded in the present study included mostly stickiness, bridges, disturbed metaphases, c-metaphases and c-anaphases, laggard chromosomes and fragments. Sticky chromosomes were found present predominantly during metaphase after the exposure duration of 24 and 36 h, then their presence decreased non significantly. 36h treatment concentrations 0.12 and 0.16% of AGRIA-MANCOZEB 80 WP and MANFIL 75 WG induced the highest stickiness observed (reaching 52% and 61% of the total respective FAC values). A positive correlation between the increased incidences of bridges and laggard chromosomes and the achievement of ana-telophase stage of meristematic root cells of onions; prolongation of time duration and addition of fungicides concentration was detected, as well.

Table 2. Genotoxic effects of Mancozeb-based fungicides on root meristem of *Allium cepa* L.

Exposure periods (h)	Tested solutions	Cc (%)	FAC ±SD (%)	FMN ±SD (%)
	NC	0	1.17±0.82	0. 0131±0.0022
24	AGRIA-MANCOZEB 80 WP	0.04	1.39±0.07^{a}	0.0139±0.0019
		0.08	2.97±0.24*^{ab}	0.0127±0.0041
		0.12	5.46±0.38**^{c}	0.0159±0.0012
		0.16	6.82±0.43**^{de}	0.0171±0.0034^{a}
	MANFIL 75 WG	0.04	2.08±0.01	0.0149±0.0039
		0.08	3.29±0.22*^{a}	0.0160±0.0018
		0.12	4.31±0.51*^{b}	0.0172±0.0039^{a}
		0.16	5.39±0.16**^{f}	0.0181±0.0024^{ab}
	DITHANE M-45 blue 72 WP	0.04	1.73±0.09	0.0118±0.0007
		0.08	2.81±0.12^{a}	0.0143±0.0032
		0.12	3.71±0.27*^{b}	0.0152±0.0014
		0.16	3.12±0.34*^{cd}	0.0169±0.0055^{a}
36	AGRIA-MANCOZEB 80 WP	0.04	3.57±0.44*^{c}	0.0164±0.0021
		0.08	6.32±0.58**^{e}	0.0143±0.0068
		0.12	9.09±0.08**^{f}	0.0211±0.0091^{b}
		0.16	10.61±0.93**^{g}	0.245±0.0043^{bc}
	MANFIL 75 WG	0.04	4.77±0.60**^{b}	0.0172±0.0031^{a}
		0.08	7.56±0.66**^{e}	0.0185±0.0054^{b}
		0.12	11.35±1.13**	0.0207±0.0044^{b}
		0.16	13.51±0.98**^{g}	0.0268±0.0079^{c}
	DITHANE M-45 blue 72 WP	0.04	3.14±0.25*^{a}	0.0146±0.0022
		0.08	4.18±0.32*^{cd}	0.0165±0.0072^{a}
		0.12	6.69±0.83**^{f}	0.0183±0.0051^{ab}
		0.16	9.29±0.75**g	0.0204±0.0064^{b}
48	AGRIA-MANCOZEB 80 WP	0.04	6.07±0.79**^{d}	0.0317±0.0056*^{cd}
		0.08	9.62±0.64**^{g}	0.0373±0.0082*^{d}
		0.12	11.51±1.06**^{fg}	0.0182±0.0061^{ab}
		0.16	14.38±1.23**^{h}i	0.0339±0.0024*^{d}
	MANFIL 75 WG	0.04	8.52±0.46**^{e}	0.0367±0.0079*^{de}
		0.08	11.17±1.19**^{g}	0.0362±0.0037*^{d}
		0.12	13.61±1.25**^{h}	0.0328±0.0045*^{d}
		0.16	12.72±1.03**^{gh}	0.0178±0.0022^{a}
	DITHANE M-45 blue 72 WP	0.04	6.58±0.39**^{bc}	0.0275±0.0031^{c}
		0.08	11.03±0.58**^{f}	0.0309±0.0098^{cd}
		0.12	10.46±0.96**^{f}	0.0337±0.0015*^{cd}
		0.16	10.79±1.01**^{fg}	0.0351±0.0029*^{d}

Within each column means labeled with asterisks and letters are significantly different from respective NC-s according to One-Way ANOVA test (* P<0.05; ** P<0.001) and between exposure periods and fungicide concentrations in SNK test (p<0.05). NC-negative control; FAC - frequencies of mitotic cells

with chromosomal aberrations; FMN - frequencies of interphase cells with micronuclei; SD – standard deviation.

For DITHANE lagging where present only at 48h samples for example and their frequency was notably enhanced ($P<0.05$ and $p<0.05$) as compared to NC and through 0.04-0.16% concentrations. Bridges were observed throughout all tested fungicides, time duration and concentrations, excluding 24h ones of DITHANE, constantly increasing after 48h exposure in particular. Altered display of metaphasic disturbance, c-meta- and c-anaphases were present under MANFIL and AGRIA-MANCOZEB 36h treatments, showing significant changes from 24h samples and NC. Insignificant breaks and fragments resulted in almost all 36 and 48h samples, but mainly in meristematic root cells which underwent the 48h treatment with 0.12 and 0.16% cc MANFIL solutions.

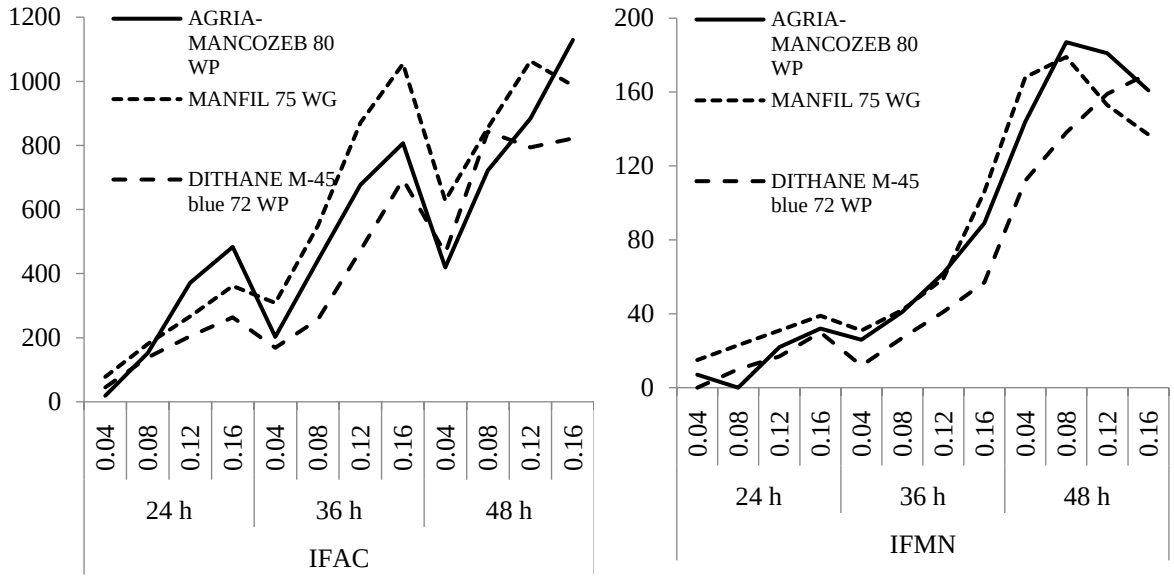

Figure 3. Comparative genotoxicity induced on *A. cepa* L. assay by selected mancozeb-based fungicides (respective cc-s in %). IFAC and IMNC -increase of frequencies of root meristem cells with chromosome aberrations and micronucleated interphase cells (expressed in % of respective NC values)

Despite the successive augmentation there was found no significant presence of micronucleated dividing cells at interphase stage in *A. cepa* roots treated for 24 and 36 h with the tested concentrations of mancozeb-based fungicides. The observed MN frequencies after 48h root treatments revealed interesting significant differences ($P<0.05$) compared to NC (0.0131% of NDC): 0.08% cc-s of AGRIA-MANCOZEB 80 WP and MANFIL 75 WG resulted the most mutagenic ones increasing up to 287 and 281% the micronuclei occurrence, contrary to the concentrations: 0.12% of AGRIA-MANCOZEB, 0.16% of MANFIL; 0.04 and 0.08% of DITHANE which did not induce significant changes from NC value. DITHANE samples inflicted the most significant FMN changes ($p<0.05$) through concentrations and time exposures, achieving a MN trebling (under 0.16% cc and 48 h treatment) in comparison with the lowest concentration (0.04%) and shortest period (24h) treatment.

4. CONCLUSIONS AND DISCUSSION

The treatment of seeds with mancozeb-based fungicides is commonly used to prevent the impairment from fungal diseases. Seed germination constitutes a decisive and crucial phase of plant life cycle, closely related to the environmental conditions. The present data demostrated limitation on seed germination of *A. cepa* L. native ecotype Drishti depending from tested fungicides' concentrations and particularly time exposure. Due to the short hife-life of Mancozeb in soil the hiperaccumulation on rhizosphere soil solutions could potentially damage radicle emergence, prevent the water and nutrient absorption, reduce the efficiency of respiratory enzyme complexes, translocation of energy containing substances to developing embryo, which might retard or inhibit the detected seed germination. Several publications highlighted similar phytotoxic effects of different fungicides and mancozeb particularly on non-target

crops physiology (Siddiqui and Ahmed, 2002; Untieldt and Blanke, 2004; Muuben et al., 2006; Dias et al., 2014; Sathees et al., 2014; Fatma et al. 2018; Monika & Kidwai, 2017; Shahid et al., 2018).
In order to assess the short-term phytotoxicity induced on higher plant assays the detection of hazards implication on the impairment of root growth processes is often applied. If the length of emerging bulbous rootlets of *A. cepa* is reduced over 55% as compared to negative control sample the rhizotoxic effects of chemical under study should be considered as strong (Fiskesjö G, 1993; Mesi & Kopliku, 2015, Bonciu E, et al. 2018; Dizdari & Bala, 2019). Moreover, if the mitotic activity of root meristematic cells is reduced below 50% and 22% of NC, it respectively demonstrates sublethal and lethal effects on the organism under study (Panda & Sahu, 1985; Antonise-Wiez D, 1990). The present data match with above mentioned statements, making evident the fact that, if common onions face into soil to high concentrations and remain exposed against solutions of mancozeb-based formulations, they can potentially undergo harmful toxicity on root growth. The recorded reduction of mitotic activity could happen because of mitotic cycle arrest at the G_2 phase and/or S-phase extension without inhibiting the synthesis of DNA (Barakat et al., 2010). In addition it should be emphasized that mancozeb-based fungicides acting by contact against fungi could seriously poison non target roots and soil organisms, since the rate of significant root growth reduction was found present even in the concentrations interval of the shortest exposure period (MANFIL 75 WG and DITHANE M-45 blue 72 WP). In the same context threshold toxicity tests are often applied to verify the point at which chemical pollutants exert significant growth damages (Fiskesjö, 1994, 1997; Mesi & Kopliku, 2013, 2015). The extrapolated EC_{50} values in the present study indicated that AGRIA-MANCOZEB 80 WP, MANFIL 75 WG and DITHANE M-45 blue 72 WP could affect the longitudinal onion root growth at such concentrations of tested mancozeb fungicide formulations which are routinely applied in Albanian agriculture. These simulating experimental data approved the effectiveness of the EC_{50} evaluation parameter to permit the use of the same assessed concentrations and time periods for potential inducement of genotoxic effects, due to the presence of sufficient meristematic cells undertaking mitosis, whatever allows to further inquire for chromosomal abnormalities. Root growth inhibition due to pesticide toxicity could be due to the suppression of root cell division/root elongation or to the extension of cell cycle (Mesi & Kopliku, 2015), followed consequently by reduced penetration of roots into the soil and inefficiency of plant to fulfil the demands for water and mineral nutrition uptake. This is sustained by the present results, with revealed a positive correlation between induction of cumulative phytotoxic effects on root length and reduced capability of root meristematic cells to be divided under mancozeb chemical stress.
According to Iqbal et al. (2019) citogenetic assays are rather appropriate to identify the harmful effects of known chemicals in different concentrations and time exposures, because the method is considered much more sensitive as compared with distinct fisical, chemical, saprobiological, radiological or simply genetic methods. The deteriorated mitotic activity of root meristematic tissue is commonly associated with the rise of chromosomal anomalies and present micronucleated cells during interphase. The phenomena reflect indeed the capability of chemicals under study to induce genotoxic effects and in some cases occur in concentrations low order than those of phytotoxicity incidences (Dizdari & Kapcari, 2017). It is a crucial purpose and a rational reason to necessarily include in such eco-toxicological studies the assessment of genotoxicity. Root meristematic tissue of *Allium cepa* is applied as an effective detector of genotoxic and mutagenic potency of environmental pollutants, especially pesticides due to the excellent correlation of data with those of the mammalian systems (Grant & Salamone, 1994; Fiskesjö, 1997; Ferretti et al., 2007; Leme & Marin-Morales, 2009; Asita & Matebesi, 2010; Pratte-Santos et. al., 2015; Bonciu et al., 2018). As mentioned by Tedesco and Laughinghouse (2012) it is one of the most efficient methods for detecting and measuring the degree of alterations in the system subjected to carcinogens/mutagens or chemical causing damage and allow to describe the effects of these damages by observing chromosomal aberrations. Such is the case of the present data which detected abundant occurrence of chromosomal aberrations and less micronuclei, induced by the treatment with tested mancozeb-based formulations and positively correlated with respective increase of concentrations and time exposure.
Predominant CA types detected in the present investigation as sticky chromosomes, bridges and fragments occur because of chromatin dysfunction (Mesi & Kopliku, 2013), while disturbed metaphase and anaphase were due to inhibition of normal spindle formation. As summarized by Boumaza et al. (2016) stickiness is inflicted by the chromosomal DNA degradation/depolymerization, DNA

condensation, sub-chromatid linkage between chromosomes, chromosomal protein adhesion, lead par consequence to cell death, due to considerable level of genotoxicity induced by tested mancozeb fungicides. Additionally, the abundant presence of anaphase bridges particularly at the longest time treatment and highest concentrations applied documents the potential clastogenic effects of active ingredient mancozeb on *A. cepa* roots. Anaphase bridges can potentially lead to chromosomal breaks found present mostly as fragments. The chromosome lagging detected in significant percentages of total FAC in the Dithane highest concentration is usually induced by a weak c-mitotic effect and indicate relevant risk of aneuploidy (Amin, 2002). Chromosomal breakage, lagging or aneuploidy often induce the formation of micronuclei, bodies made of chromatin material and located in cytoplasm mainly during interphase. This phenomenon indicates potential mutagenic effects of tested chemicals and respective concentrations, being predictive even for cancer (Maluszynska and Juchimiuk, 2005, Ma et al. 2005; Dizdari & Bala, 2019). Similar DNA damage and apoptosis by mancozeb have been also reported Calviello et al. (2006) in rats.

Concluding the increasing range of morpho- and genotoxicity induced by different concentrations and duration treatments on *A. cepa* bulbous roots with tested fungicides resulted as follow: DITHANE M-45 blue 72 WP < AGRIA-MANCOZEB 80 WP < MANFIL 75 WG. To our knowledge no previous research investigations according the toxic activity of trade formulations of mancozeb-based fungicides used in Albania on plants and particularly on an Albanian native ecotype of common onion was previously reported. The current data should serve as a real alert according to the purity of their active ingredient and production origin, uncontrolled field appliance of doses and proper periods, which can bring to substantial toxic concentrations of analyzed fungicides, engendering as significant hazards to each ecosystem component and human heath as dietary recipient in food chains.

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