Plant Archives Vol. 22, No. 1, 2022 pp. 261-267 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2022.v22.no1.041

EFFECT OF THE PESTICIDE TREATMENT DOSE ON THE BIOCHEMICAL RESPONSE OF THE POTATO PLANTS (*SOLANUM TUBEROSUM* L.)

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(Date of Receiving : 25-11-2021; Date of Acceptance : 02-03-2022)

ABSTRACT

In order to enhance and protect crop yields from different pests, the use of pesticides has increased. In Algeria, most often farmers do not respect the recommended dose of pesticides. The aim of this study is to investigate the dose effect of applying pesticides in field conditions at standard and double doses (agronomic dose, AD; agronomic double dose, ADD) on the morphological and biochemical responses of potato plants (*Solanum tuberosum* L.). Our result showed significant effect of pesticide application on the biochemical and antioxidant properties of the plant. Higher contents of phenolic and flavonoid were noticed in plants treated with AD and ADD. The accumulation of malondialdehyde increases lipid peroxidation due to the excessive reactive oxygen species production. The significant increase of catalase, ascorbate peroxidase and phenylalanine ammonia lyase activities were recorded in the final growth stage compared with control. AD increases proline content in the final growth stage, but a decrease for chlorophylls and carotenoids. In addition, the decrease in thiols level was observed for both growth stages. In conclusion, the results demonstrated that treatments with pesticides at higher doses than recommended caused oxidative disturbances in potato plants and lead to an enhanced capacity of antioxidant enzymes.

Keywords: Agronomic dose; growth stage; oxidative stress; pesticides; potato

Introduction

Pesticide contamination in agricultural soil is one of the most severe ecological problems due to intensive agriculture. The pesticides used made it possible to increase agricultural production, but a close dependence on these agrochemicals has developed. Their excessive and persistent use induced damage in farmland and causes serious soil pollution and deteriorated soil quality. Application of pesticides causes toxicity to plants, which can be observed in the form of chlorosis, necrosis, stunting, burns and twisting of leaves (Sharma *et al*., 2018a). Therefore, new alternatives and less harmful strategies need to be established for the control and treatment of infectious diseases caused by phytopathogens (Morales-Irigoyen *et al*., 2018). The accumulation of fungicides and herbicides in the soil induces negative effects on the microbial flora of the soil and consequently, there will be a toxicity of residues on all living organisms. The use of several pesticides on the same crop will have consequences on soil health and its function. The microbial degradation constitutes the primary route of metribuzin herbicide degradation; this is related to soil environment, particularly by level of organic matter (Vinther *et al*., 2008). However, the chlorothalonil disrupts the biological diversity of soil microorganisms, and negatively affects the microbiological activity (Baćmaga *et al*., 2018). It belongs to the group of chlorinated benzonitriles. Chlorothalonil affects the abundance of genes involved in nitrogen cycling (Li *et al*., 2017). Famoxadone-cymoxanil used in agriculture led to developmental toxicity in the living organisms. Cheng *et al*. (2020) suggested that famoxadone-cymoxanil could induce developmental toxicity, immunotoxicity and neurobehavioral toxicity in zebrafish larvae.

Potato (*Solanum tuberosum* L.) is one of the most consumed vegetables in the world. It has a significant economic impact as it is classified as the fourth crop grown and consumed after maize, wheat and rice due to its high nutritive value and yield productivity to soil occupation ratio in comparison with other crops (FAO 2017). It is considered as a source of nutrients, vitamins, numerous macro-elements and microelements and several polyphenols compounds. Potato is exposed to abiotic and biotic stresses as viruses, fungi, oomycetes, bacteria, nematodes, parasitic weeds and insects (Makarova *et al*., 2018). Potato cultivation can be affected not only by pathogens, but also by an inadequate pesticide management system, and one of the environmental problems is the overdose of pesticides.

The objective of this study was to investigate the effect of two doses (agronomic dose of pesticide, AD; and agronomic double dose of pesticide, ADD) of several pesticide treatments on the biochemical parameters responses of the potato (*Solanum tuberosum* L.) plants.

Materials and Methods

Plant material, culture condition and treatment

This study was conducted using commercial potato (*Solanum tuberosum* L., variety Sylvana). Potato tubers were planted by hand in mid-January 2019 and harvested in May 2019 at the stage of maturity. A plot of about 44.2 m^2 was exploited to test the effect of two doses of pesticides on biochemical parameters of the potato plants. This plot was distributed in three micro plots about 5.2 m^2 each spaced out between them by 1 m. The first is cultivated untreated, it was used as a control, the second micro plot is cultivated and treated with agronomic dose of pesticide (AD), and the third micro plot was cultivated and treated with an agronomic double dose of pesticide (ADD). Phytosanitary treatments were applied by spraying, first with herbicide then fungicides and insecticides. Every twelve days between two fungicides or two insecticides, and four days between a fungicide and insecticide. The chemicals used are listed in Table 1. The leaves were harvested in the two stages, initial stage of growing and in the final stage of maturation. They were stored under cold condition in order to perform the biochemical analysis.

Measurement of growth parameters

Ten plants were randomly collected from the experimental unit for determination of biometric parameters: leaf numbers, the number of aerial stems/plant and plant height (cm). The number of tubers/ plant, total yield/plant (kg) and total yield/hectare (ton) were also calculated in the final stage of harvesting for both doses AD and ADD.

Determination of chlorophyll content

A leaf sample (500 mg) was mashed in a mortar and pestle with 80% acetone (v/v), the extract was filtered and centrifuged in sealed tubes at 15000 xg for 5 min. The absorbance was recorded at 470, 645 and 663 nm. The chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Cx+c) contents were calculated using the formulae of Lichtenthaler and Buschmann (2001):

$$\text{Chl a } (\mu g/mL) = 12.25\ A_{663} - 2.79\ A_{645}$$

$$\text{Chl b } (\mu g/mL) = 21.50A_{645} - 5.10A_{663}$$

$$\text{C (x+c) } (\mu g/mL) = (1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198$$

$$\text{Total chlorophyll } (\mu g/mL) = (20.2 \times DO_{645}) + (8.02 \times DO_{663})$$

Determination of total phenolic, flavonoid and soluble sugar content

The total phenolic content was determined by the method of Singleton and Rossi (1965). Flavonoids content of the extracts were determined using the protocol given by Zhishen *et al*. (1999). Soluble sugar content was determined by the method of Dubois *et al*. (1956).

Activities of antioxidant enzymes

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to the method described by Nakano and Asada (1981). The catalase (CAT, EC 1.11.1.6) activity was determined by the decrease of the absorbance at 240 nm during 1 min as H_2O_2 was consumed according to the method of Aebi (1984). Phenylalanine ammonia lyase (PAL, EC. 4.3.1.5) activity was estimated using the method of Beaudoin-Eagan and Thorpe (1985). The protein concentration was determined according to Bradford (1976) method.

Antioxidant capacities

The diphenylpicrylhydrazyl radical (DPPH) was estimated according to Shimada *et al*. (1992). The Ferric reducing antioxidant power (FRAP) assay was applied by Rosales *et al*. (2006) to determine the anti-oxidative capacity of potato. The FRAP assay was determined from a standard curve of ammonium ferrous sulfate, and expressed as ferrous equivalent in µM.

Determination of malondialdehyde (MDA) content and hydrogen peroxide level

MDA was estimated in plant tissues according to the protocol described by Heath and Packer (1968). Accumulation of H_2O_2 in leaves was detected according to the method of Velikova *et al*. (2000)

Measurement of proline content and total thiols

Proline content was estimated as described by Bates *et al*. (1973). Total thiols content were determinate by Sedlak and Lindsay (1968) method.

Statistical analysis

All statistical analysis was performed with SPSS package (IBM SPSS Statistics, version 22.0) using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for statistical comparisons. The value of $p<0.05$ represent significant difference.

Results

Effect of pesticides on the growth parameters

Results in Table 2 showed no significant effect of different doses of pesticides on all measured vegetative growth, plant height, leaf area (cm^2) and number of aerial stems/plant compared with untreated control. Measurements of number of tubers/ plant and total yield / plant (Kg) and total yield/hectare (ton) of potato plants treated with AD and ADD showed a significant ($p<0.05$) increase of potato yields for the AD and ADD compared with control (Table 3).

Effect of pesticides application on the photosynthetic pigments content

The effect of pesticides on chlorophyll a, b, total and carotenoids was tested on leaves of potato plants. Photosynthetic pigments are sensitive to the effects of pesticides; the chlorophyll (a) content decreased by 19% and 25% for AD and ADD respectively compared with control in the initial growth stage, and decreased for all treatment in the final growth stage (Table 4). As a response to stress of pesticides, a significant reduction of chlorophyll (b) was shown with ADD by 33% in the initial stage compared with control (Table 4). Pesticide concentrations (AD and ADD) application showed decreases in the carotenoid content by 14 % and 32% for AD and ADD respectively compared to the control of the initial stage. However, the trend of carotenoids was increased significantly for both doses AD and ADD compared with control in the final growth stage. All the pesticides with different doses as AD and ADD reduced significantly the concentration of chlorophyll total in the initial growth stage (Table 4).

Effect of pesticides application on the total flavonoid, phenolic and soluble sugar contents

In this study, the flavonoid contents were increased in both stages. We noticed that the flavonoid was increased by 80% and 160% for the AD and ADD respectively in the initial growth stage compared with control and by 123 % and 293 % for both doses, respectively in the final growth stage compared with control, so that significant increased activity was observed in the final growth stage for AD and ADD (Table 4). The results of the present study revealed that the level of total phenolic compounds enhanced in both stages with 39% and 107% for the AD and ADD respectively of the initial stage compared with control, and with 22% and 60% for AD and ADD respectively of the final stage. However, these results showed significant levels of phenolic in plants treated with ADD of the two stages (Table 4). Soluble sugar contents showed a significant increase in the leaves of plants treated by standard and double agronomic dose at the final growth stage compared with initial stage, and no significant difference in the same growth stage.

Effect of pesticides application on the antioxidant enzyme activity

APX activity exhibited a significant variation trend of AD and ADDP in the both stages compared with control. A significant increase activity was observed in the final growth stage in AD and ADD by 130%, 175% respectively (Table 5). The CAT activity exhibited similar response upon pesticide exposure in both the growth stages. Pesticides enhanced CAT activity in the final growth stage by 67% and 122% for the AD and ADD respectively compared with control. The result of PAL activity recorded high values in ADD by 127% in the initial stage and 100 % in the final stage (Table 5).

Effect of pesticides application on the antioxidant capacities of potato

In both assays (DPPH and FRAP) the antioxidant capacity in leaves was significantly higher in final stage compared with control. However, no significant activity was notified for the two doses of the initial stage (Table 6). The results of ferric reducing antioxidant power (FRAP) decreased within treatments AD and ADD by 7% and 11% respectively for initial growth stage, but increased in the final stage with a significant antioxidant capacity recorded at an agronomic double dose of pesticide (ADD) (Table 6).

Effect of pesticides application on the malondialdehyde (MDA), hydrogen peroxide (H_2O_2) level, proline content and total thiols.

The degree of lipid peroxidation was estimated by the MDA assay in plant's leaves. The highest amount of MDA level was found in plants treated with both doses in initial growth stage. However, a significant increase was recorded in H_2O_2 level in final growth stage (Table 7). Proline is considered as a biochemical marker of the response of plants to abiotic stress. In our study, similar proline values were measured in plant leaves treated with the different doses of pesticides for the initial growth stage. However, the final stage showed the high proline value for the ADD. Our result showed no significant change of the thiols content in both stages, and significant change of hydrogen peroxide levels between initial and final growth stage (Table 7).

Discussion

The present study was designed to assess the effects of two doses of pesticide treatments at the recommended agronomic dose and double dose on the morphological and biochemical responses of potato (*Solanum tuberosum* L.). The results of all measured vegetative growth showed that the growth was not affected by the two doses of pesticide. The plants height showed no significant effect in initial growth stage under both pesticides applications in accordance with Wu *et al*. (2004). However, a significant effect was recorded at the final growth stage compared with control. Similar results were reported by Sharma (2016) who mentioned that cypermethrin pesticides application affect significantly the growth and height of plants so that the lower concentration of cypermethrin application stimulate the height of plants, but at a higher concentrations inhibits all the growth parameters. In contrast, Gafar *et al*. (2018) reported that both Folimat 800 and Icaros pesticides applied at recommended and excessive dose increased carrot vegetative growth, but without difference between both doses. In this study, potato yield was no significantly affected by application of both low and high dose of pesticides, but Glover-Amengor and Tetteh (2008) reported that the doses of pesticides used (unden and diathane applications) affect crops by increasing yield at a lower concentrations and decreases at high concentrations. It is well noted that yield of plants depends upon the nutrient uptake through the root, but the dose of pesticide can influenced the function and soil health (Lupwayi *et al*., 2010). We have demonstrated that the exposure of potato plants to high dose of pesticides induce reactive oxygen species (ROS) production and enhanced the antioxidant enzymes activities. ROS causes oxidative stress to plants under pesticide application (Sharma *et al*., 2018b).

Photosynthesis is an important process for the growth and development of plants. In this study, the photosynthetic pigment contents were disturbed under pesticides treatments, but this exposure enhanced total phenol contents and flavonoids. Xia *et al*. (2006) reported that pesticides-induced reduction in the photosynthetic efficiency of plants by reducing stomata conductance, and the generation of oxidative stress; and affect various metabolic processes of plants as the effective quantum yield, enzyme activities, root growth and biosynthesis of pigments (Sharma *et al*., 2016). The decreased chlorophyll and carotenoid content in the initial growth stage of potato leaves is the early symptoms of pesticide toxicity. Parween *et al*. (2016) reported that application of higher dose of fungicide captan reduces the chlorophyll and carotenoid content in plants. Under environmental stress, such as pesticide exposure, phenolic and flavonoid compounds scavenger ROS excess and protect plants from oxidative stress (Berni *et al*., 2019; Pourcel *et al*., 2007) In the current study, the increase of flavonoids and total phenol content may be due to the increase in PAL activity (Kováčik *et al*., 2007), indicate that pesticides stimulated the phenylpropanoids pathway while the accumulation of this secondary metabolite is an adaptive strategy and protective mechanism to deal with stress caused ROS over production (Gill and Tuteja, 2010). Our results for polyphenol contents in potato leaves (cultivar Sylvana) are lower than those reported by Zarzecka *et al*. (2019) using other cultivars (Bartek, Gawin, and Honorata). Sugars are considered as biochemical molecules that are responsible of providing enough energy to plants and govern

photosynthesis, flowering, senescence, respiration, and seed germination. Our results showed that sugar content increased no significantly in the final growth stage under pesticides treatments. In accordance with the results reported by Gugała *et al*. (2013) that herbicides had no effect on total sugars. Ascorbate peroxidase (APX) is thought to play important role in scavenging ROS and protecting cells in plants. Our findings showed the increased APX activity in both agronomic doses of pesticide (AD) and agronomic double dose of pesticide (ADD) in the final stage of potato plants. This result is in accordance with Shakir *et al*. (2018) who reported that pesticides induced APX activity in the shoot of tomato plants treated with emamectin, cypermethrin and imidacloprid. Similar levels of CAT activity were detected in the leaves of plants for the initial growth stage. On the other hand, a significant increase in CAT activity was observed in the leaves of plants treated under ADD at the final stage. In addition, the activities of antioxidant enzymes such as catalase have improved in response to pesticides applied. Oxidative stress caused by the application of pesticides has been found to be somewhat correlated with the increase in the concentration of pesticides. Parween *et al*. (2012) demonstrated that the foliar application of chlorpyriphos insecticide in *Vigna radiata* L. enhanced CAT activity. Phenylalanine ammonia-lyase (PAL) is the rate limiting enzymes in phenolic compounds production through phenylpropanoids pathway (Kováčik *et al*., 2007). PAL activity increased significantly and enhances tolerance to pesticide stress by the accumulation of secondary metabolites, especially flavonoids as effective ROS scavenger.

The free radical scavenging capacity (DPPH) and the ferric reducing antioxidant potential (FRAP) were used to assess the potential antioxidant and antiradical activity of the potato. This activity increased significantly for both AD and ADD doses of the final growth stage compared to control. Our results were in accordance with studies from Radwan (2012), who showed that clethodim caused a significant increase in total antioxidant activity (DPPH) in leaves of Maize (*Zea mays* L.). In this current study, the activity of DPPH and FRAP correlates with the content of secondary metabolites, including flavonoids. Improvements in the antioxidant capacity of potato leaves exposed to pesticides can be attributed to higher flavonoid content, indicating that the potato had stronger antioxidant capacity to eliminate ROS and attenuate oxidative damage. The increased accumulation of flavonoids and a higher antioxidant capacity is a biochemical adaptation used by potato to avoid oxidative damage, and could be improved tolerance to pesticides stress.

Oxidative damage parameters were represented as lipid peroxidation products (MDA biomarker), and reactive oxygen species (hydrogen peroxide). These results showed that treatment with both simple and double dose of pesticides generates increased the lipid peroxidation level. These results are in agreement with the findings reported by Kaya and Doganlar (2016). However, the hydrogen peroxide level increases significantly only on the final growth stage. Proline osmo-protectant is also known for its antioxidant activity in plants submitted to abiotic stress. It produced and accumulates by plants under abiotic stress and act essential roles to increase stress tolerance. Low-molecular-weight (thiols) plays also a key role in the tolerance of plants to abiotic stress such as pesticides. Our results showed high level of proline in plants treated by ADD at the final growth stage, and no significant decrease in the contents of thiols during exposure to pesticides in both growth stages.

Conclusion

In the present study, we have demonstrated that the exposure of potato plants to high dose of pesticides induces ROS production, and enhanced the antioxidant enzyme activities and accumulating soluble proline. The pesticide application induced increasing of phenolic and flavonoid compounds which were correlated with stronger antioxidant potential, and soluble sugar contents. These biochemical responses allow the development of effective strategies to reduce the negative effect of pesticides on agricultural production, plant growth and human health. Pesticide applications at high dose increase soil chemical load and induce effects on soil microbial communities and disturbance on the function of soil organisms. Consequently, a decrease in soil productivity and residue accumulation in plants will be obtained. Our results made it possible to indicate the response mechanism of plants to pesticide stress in order to understand the effect of phytotoxicity on crops.

Table 1 : Pesticide treatments of potato under field conditions

Active ingredient	Manufacture recommended use rate
Abamectin (18 g/L)	50 mL/ha
Famoxadone (22.5 %) + Cymoxanil (30%)	400 g/ha
Metribuzin	450-650 g/ha
Chlorothalonil (500 g/L)	2L/ha
Chlorothalonil (500 g/L) + Metalaxyl (36.3 g/L)	2L/ha

Table 2 : Effect of different doses of pesticides on leaf area (cm^2), number of aerial stems/plant and plant height (cm) for the initial and final growth stage of potato plants.

	Initial growth stage			Final growth stage		
	Leaf area (cm^2)	Number of aerial stems/ plant	Plant height (cm)	Leaf area (cm^2)	Number of aerial stems/plant	Plant height (cm)
Control	25.12 ± 6.25^a	2.66 ± 0.25^a	13.08 ± 2.79^a	49.22 ± 8.86^b	2.66 ± 0.25^b	16.08 ± 2.89^b
AD	27.74 ± 4.17^a	2.53 ± 0.55^a	17.55 ± 1.53^a	52.94 ± 5.73^b	2.53 ± 0.55^b	22.55 ± 1.53^c
ADD	24.97 ± 2.88^a	2.66 ± 0.25^a	16.95 ± 1.30^a	49.12 ± 4.03^b	2.66 ± 0.25^b	21.95 ± 1.30^c

AD, agronomic dose of pesticide; ADD, agronomic double dose of pesticide.
Each value is the mean± SD of 3 replicates. Values in the columns followed by different small letters are significantly different at $p< 0.05$.

Table 3 : Effect of different doses of pesticides on number of tubers/ plant and total yield/plant (kg) and total yield/hectare (t/ha) of potato

Parameters	Control	AD	ADD
Tubers number/plant	6.35 ± 0.91^{a}	8.38 ± 0.24^{b}	7.49 ± 0.99^{ab}
Tubers weight/plant (kg)	0.47 ± 0.03^{a}	1.08 ± 0.01^{b}	0.86 ± 0.03^{c}
Total yield/hectare (t/ha)	13.59 ± 1.03^{a}	28.81 ± 3.32^{b}	24.85 ± 1.10^{b}

AD, agronomic dose of pesticide; ADD, agronomic double dose of pesticide.
Each value is the mean± SD of 3 replicates. Same letters indicate no significant differences at $P < 0.05$

Table 4 : Effects of pesticides on contents of chlorophyll a, b, carotenoids and total chlorophyll, phenolic compounds contents and soluble sugar of potato plants.

Parameters	Initial growth stage			Final growth stage		
	Control	AD	ADD	Control	AD	ADD
Chlorophyll a ($\mu g.g^{-1}$ FW)	767 ± 6^{a}	621 ± 82^{b}	574 ± 98^{b}	282 ± 29^{c}	240 ± 26^{c}	301 ± 40^{c}
Chlorophyll b ($\mu g.g^{-1}$ FW)	1384 ± 17^{a}	1038 ± 296^{b}	916 ± 214^{b}	202 ± 15^{c}	217 ± 21^{c}	221 ± 151^{c}
Carotenoids ($mg.g^{-1}$ FW)	88.32 ± 1.13^{a}	76.32 ± 7.81^{a}	60.56 ± 23.34^{a}	6.82 ± 1.05^{b}	27.79 ± 0.17^{c}	11.98 ± 3.91^{d}
Total chlorophyll ($\mu g.g^{-1}$ FW)	2180 ± 11^{a}	1766 ± 256^{b}	1512 ± 317^{b}	225 ± 78^{d}	285 ± 87^{d}	281 ± 38^{d}
Flavonoid (µg EQ/g FW)	22.05 ± 10.39^{a}	39.70 ± 22.87^{a}	57.35 ± 14.55^{a}	35.58 ± 4.57^{b}	79.41 ± 2.94^{c}	140.19 ± 28.56^{d}
Total phenol (mg GAE/g FW)	17.90 ± 3.65^{a}	24.96 ± 9.86^{ab}	37.20 ± 5.24^{b}	3.43 ± 0.32^{c}	4.20 ± 1.01^{cd}	5.50 ± 0.65^{d}
Soluble sugar (g /g FW)	0.061 ± 0.009^{a}	0.137 ± 0.059^{a}	0.081 ± 0.001^{a}	0.245 ± 0.001^{b}	0.249 ± 0.010^{b}	0.243 ± 0.002^{b}

AD, agronomic dose of pesticide; ADD, agronomic double dose of pesticide; mg GAE/g FW, mg of gallic acid equivalents per gram of fresh weight; µg EQ/g FW, µg of equivalent quercetin per gram of fresh weight. Each value is the mean ± SD of 3 replicates. Same letters indicate no significant differences at $p < 0.05$ and different small letters are significantly different at $p< 0.05$

Table 5 : Effects of pesticides on the antioxidants enzymes of potato plants

Enzymatic activities ($mM.min^{-1}.mg^{-1}$ of protein)	Initial growth stage			Final growth stage		
	Control	AD	ADD	Control	AD	ADD
CAT	0.006 ± 0.001^{a}	0.007 ± 0.001^{a}	0.017 ± 0.001^{b}	0.006 ± 0.001^{a}	0.01 ± 0.001^{cd}	0.013 ± 0.002^{d}
APX	0.072 ± 0.014^{a}	0.039 ± 0.005^{b}	0.099 ± 0.010^{a}	0.033 ± 0.005^{b}	0.077 ± 0.002^{a}	0.092 ± 0.0003^{c}
PAL	1.42 ± 0.30^{a}	1.39 ± 0.27^{a}	3.24 ± 0.58^{b}	1.10 ± 0.35^{a}	2.23 ± 0.21^{c}	$2.13 \pm 0.50^{b,c}$

AD, agronomic dose of pesticide; ADD, agronomic double dose of pesticide; CAT, catalase; APX, ascorbate peroxidase; PAL, phenylalanine ammonia lyase. Values followed by different small letters are significantly different at $p< 0.05$

Table 6 : Free radical scavenging capacity (DPPH) and FRAP activity of potato plants

Antioxidant capacities	Initial growth stage			Final growth stage		
	Control	AD	ADD	Control	AD	ADD
DPPH inhibition (%)	36.65 ± 1.44^{a}	30.80 ± 4.69^{a}	38.38 ± 2.17^{a}	11.84 ± 8.71^{b}	49.92 ± 7.13^{c}	52.13 ± 2.84^{c}
FRAP activity (µM ferrous equivalent)	82 ± 22.62^{a}	76.33 ± 21.12^{a}	73 ± 14.14^{a}	220.33 ± 25.38^{b}	255 ± 47.00^{bc}	333.33 ± 44.29^{c}

AD, agronomic dose; ADD, agronomic double dose.
Values followed by different small letters are significantly different at $p< 0.05$

Table 7 : Effects of pesticides application on the malondialdehyde (MDA) content, hydrogen peroxide level, proline and total thiol contents of potato plants

Non enzymatic Antioxidants	Initial growth stage			Final growth stage		
	Control	AD	ADD	Control	AD	ADD
Thiols (mM)	43.60 ± 7.38^{a}	35.88 ± 1.97^{a}	38.45 ± 12.37^{a}	84.22 ± 22.20^{b}	83.57 ± 28.99^{b}	83.89 ± 8.32^{b}
Proline (mol/g FW)	4.59 ± 0.92^{a}	4.60 ± 0.96^{a}	4.69 ± 0.92^{a}	9.69 ± 0.83^{b}	9.28 ± 1.40^{b}	$13.03 \pm 1,70^{c}$
MDA (µmol. g FW^{-1})	0.08 ± 0.03^{a}	0.28 ± 0.06^{b}	0.82 ± 0.12^{c}	0.511 ± 0.03^{d}	0.54 ± 0.16^{d}	0.66 ± 0.10^{d}
Hydrogen peroxide level (µmol H_2O_2. g FW^{-1})	2.66 ± 0.41^{a}	3.14 ± 0.55^{a}	2.08 ± 0.30^{a}	1.83 ± 0.08^{b}	2.32 ± 0.09^{c}	2.29 ± 0.11^{c}

AD, agronomic dose; ADD, agronomic double dose; mol/g FW, mol per gram of fresh weight.
Values followed by the same letter were not significantly different at the 5% level of significance.

Conflict Of Interest

The authors declare no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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