African Journal of Agricultural Research Vol. 6(31), pp. 6563-6571, 19 December, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.663
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

Endosulfan induced alterations in physiological responses in *Lycopersicum esculentum* seeds during germination

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Accepted 26 June, 2011

The impact of endosulfan (an organochlorine insecticide), at four concentrations (0.015, 0.03, 0.045 g/L and its recommended field rate 0.06 g/L) on some morphological and biochemical parameters was investigated during the germination process of tomato seeds. The seeds germinating in presence of endosulfan had less and later emergence than the control seeds; the insecticide caused a decrease in both roots and shoots length. Protein patterns showed an over expression in all seeds exposed to endosulfan treatment during germination. Even at low concentration (0.015 g/L), six fold higher esterase activity was recorded compared to control. The same effect was shown on protease activity which was three fold greater compared to control at concentration of 0.06 g/L; however, a significant inhibition of α-amylase activity was detected. At concentration of 0.015 g/L, the activity was around 60% compared to control. On the other hand, an increase in proline levels was observed in response to endosulfan application.

Key words: Insecticide, endosulfan, tomato, seed germination.

INTRODUCTION

Crop plants are attacked by all kinds of pests and pathogens such as insects, nematodes, fungi, bacteria and viruses (Robert and Giles, 1980). Pesticides are chemicals used to protect crops from insects, weeds and fungal attack and rodents. The use of pesticides has enabled the production of a sufficient quantity of agricultural produce (Robert and Giles, 1980). Chemical pest control has a central place in modern agriculture. Most chemicals used as pesticides however are toxic and the major argument against their use is the health risk factor and the danger of environmental pollution (Robert and Giles, 1980; Kilmer et al., 2001).

Germination in plants could be described as the origination of a new organism from a pre-existing embryo in the seed. Various dynamic processes triggered by hydration signals result in active cell division and elongation and, ultimately, embryo emergence through the seed coat (Bewley and Black, 1985). The germination process starts with seed imbibition and ends with the protrusion of the embryonic axis (the radicle) through the enclosing tissues (Bewley and Black, 1985). The process is influenced by available soil moisture, soil temperature, and the nature of the soil surrounding the seed or residual pesticides that may be present in the soil (Dwain Meyer, 1999). During the germination process, storage proteins are hydrolysed and amino acids are released

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Abbreviations: EDTA, Ethylene diamine tetraacetic acid; **SDS**, sodium dodecyl sulfate; **NaCl**, sodium chloride; **TCA**, trichloroacetate; **SDs**, standard deviations; **ANOVA**, analysis of variance; **HSP 70**, Heat stock proteins 70; **GA**, gibberellic acid; **H^+,** hydrogen ion.

(Lea and Joy, 1983) such as proline among others that may be catabolized during the early stages of germination to afford energy to the new formed tissue (Below et al., 2000; Nakashima et al., 1998; Goldraij and Polacco, 1999, 2000).

Proline is known to increase during seed germination (Lea and Joy, 1983) and to take part in structural proteins that participate in the edification of cell wall of young tissues (Hare and Cress, 1997; Nanjo et al., 1999). Proline is also known to accumulate in plants subjected to unfavourable environmental conditions (Aspinall et al., 1981). This amino acid accumulates in many plant species under a broad range of stress conditions such as water shortage, salinity, xenobiotics, extreme temperatures among others (Aspinall et al., 1981; Mansour, 2000; Bordjiba and Ketif , 2009).

Alpha amylases catalyze the hydrolysis of a-D-(1,4)-glucan linkages in starch components, glycogen and various other related carbohydrates. They constitute a widely distributed family of enzymes found in microorganisms, plants and animals (Strobl et al., 1998). Their levels in plants are altered by xenobiotics such as heavy metals (Mihoub et al., 2005) and pesticides (Wilkinson, 1988; Mamdouh et al., 1998; Sabale and Misal, 2000).

General esterases are a large and diverse group of hydrolases that hydrolyze numerous substrates including esters and some non-ester compounds (Walker et al., 1983). Esterases have long been used in plants as biomarkers for genotyping in zymogen analysis of crops and weeds (Wouters and Booy, 2000) as well as markers for cell viability (Steward et al., 1999). Feng et al. (1995) assigned to esterases the role of inactivation of the herbicide thiazopyr. Cumminsa et al. (2001) carried out a study dealing with the contribution of esterases to regulating the activity of pesticides in wheat. For this purpose, they have successfully purified and characterized a *p*-nitrophenyl acetate-hydrolysing esterase from wheat shoots. Few studies have been undertaken with the interactions between plants and insecticides. Regardless of this fundamental lack of understanding, we were interested in studying the effect of the insecticide endosulfan during seed germination. Tomato was selected as target plant because of its worldwide economic importance and its fast germination and growth. In this study, we have evaluated the effects of endosulfan on morphological parameters such as percentage of germination, shoot and root elongation and biochemical ones such as proline, proteins, α-amylase, esterase and protease activities.

MATERIALS AND METHODS

Chemical solutions

Endosulfan is one of the most used insecticides in the north of Morocco; four solutions (which correspond to dilutions of the dose of fields) were prepared 0.015, 0.030, 0.045 and 0.06 g/L (dose of fields), with demineralised water; pH was approximately seven, the same pH as the control. The chemical prosulfan used in the experiments is produced by the CEQUISA using endosulfan at 35 g/L as active compound.

Plant material and germination process

Tomato (*Lycopersicum esculentum*) seeds were surface-sterilized in 10% commercial bleach with stirring for 5 min followed by extensive washing in sterile-distilled water. Batches of 50 seeds of tomato were germinated in Petri dishes (diameter 9 cm) on top of two layers of filter paper moistened with 6 ml of either distilled water or insecticide solutions at concentrations of 0.015, 0.030, 0.045 and 0.06 g/L (the later represents the fields dose) of insecticides (Endosulfan prosulfan), and maintained in a growth chamber in darkness at 25°C. At various stages of tomato germination, seeds of each replicate were collected for the germination evaluation and embryonic roots and shoot length measurements. Four replicates were performed; germination time was determined as the time of rupture of the seed coats and the emergence of the radicle; seedlings were grown for up to a maximum of 6 days.

Proteins measurement

Plantlets were homogenized in 0.1 MTris-HCl, pH 7.2 and proteins were quantified using the Bradford method (Bradford, 1976) using bovine serum albumin as standard.

Proline measurement

Proline was quantified as described in Bates et al. (1973), with some modifications; samples from different treatments and control were homogenized in 1 ml 3% aqueous sulphosalicylic acid and centrifuged at 9 000 g. 1 ml of supernatant was reacted with 1 ml ninhydrin acid and 1 ml glacial acetic acid in a test tube for 60 min at 100°C. The reaction was stopped in an ice bath. The mixture was extracted with 4 ml toluene and mixed with a test-tube stirrer for 15 s. The chromophore containing toluene was separated, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank.

Esterase measurement

The activity of esterase was evaluated by the method of Karoly (Karoly et al., 1996) with some modifications. 100 mg of germinating seeds were homogenized in 100 mM phosphate buffer (pH 7.0) containing 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM PMSF and 0.5% PVP. The mixture was centrifuged at 9000 g for 20 min. The supernatant was used as enzyme extracts to determine enzyme activity. Esterase activity was determined by preparing a mixture of 1 ml of 0.6 mM β-naphthyl acetate (2-NA) in 0.1 M phosphate buffer , pH 7 and 1 ml of supernatant and incubated at 30°C for 15 min ; the reaction was quenched by adding of 240 µl of a dye solution prepared mixing fast garnet with sodium dodecyl sulfate (SDS). Naphthol-fast blue complex was formed and measured after 15 min at 492 nm, β-naphthol was used as standard.

Alpha amylase activity measurements

The activity of α-amylase was evaluated according to Valencia methods (Valencia et al., 2000) with some modifications. The enzyme activity was assayed by measuring the reducing sugar released during

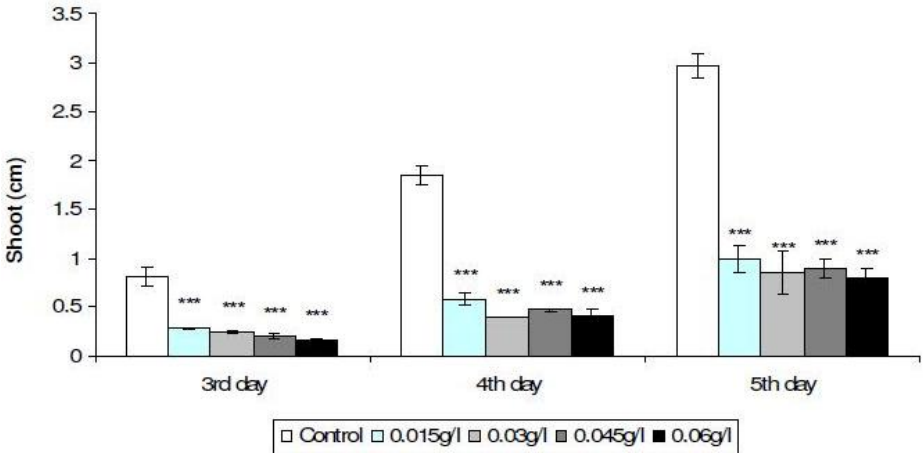

Figure 1. The effect of endosulfan on shoot length of tomato seedlings. Each value represents the mean + SE of three replications. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively.

the reaction, using starch as a substrate. Alpha amylase was extracted by homogenizing 100 mg of plant material in 0,1M phosphate buffers (pH 7.0) and 0,9 ml of 100 mM sodium citrate buffer (pH 5.0); the extract was centrifuged at 9000 g for 20 min. The reaction mixture contained 0,1ml of 0.5% soluble starch in 0.8 ml of 100 mM sodium citrate buffer (pH 5.0) and 0.1 ml of enzyme solution. After incubating for 15 min at 37°C, the suspension was stained with lugol and centrifuged at 4000 g during 15 min. Activity was determined by measuring the reducing sugar content at an absorbency of 580 nm.

Protease measurement

The azocaseinolytic activity was determined by the modified Tomarelli's method (Tomarelli et al., 1949). The reaction mixtures consisted of 2 ml of partially purified culture medium and 1 ml of 2% (w/v) azocasein dissolved in 0.9% sodium chloride (NaCl) and in 0.05 M phosphate buffer (pH 6.8), and 0.2 M HCl-tris buffer (pH 8). After 15 min of incubation at 30°C, the reaction was arrested by adding 20% (w/v) trichloroacetate (TCA); precipitation and centrifugation (3000 g, 20 min) took place at 4°C, then the absorbance was read at 440 nm. In the control, TCA was added to the culture medium prior to azocasein. One unit of protease activity was defined as the amount of enzyme that increased the absorbance by 0.1 at 440 nm per 1 h at 28°C.

Statistical analysis

Data are presented as means ± standard deviations (SDs) of three independent tomato seedlings and were analyzed by a one-way analysis of variance (ANOVA) Statistical software using Tukey HSD test (Statistical, 1997). Differences among means were considered significant with $*P \leq 0.05$, highly significant with $**P \leq 0.01$and very highly significant with $***P \leq 0.001$.

RESULTS AND DISCUSSION

Effect of insecticide on germination

Our results showed a significant decrease in tomato seeds germination rate in the presence of the insecticide and at different concentrations since the beginning of the test period compared to control. We may also suggest that the germination and generally the first stages of the growth process were retarded in the treated seeds. Similar declines in seed germination rate have been reported in the literature with other pesticides; likewise, paraquat dichloride decreased significantly the seed germination rate in *Typha latifolia* to half at concentration of 1.0 mg/L (Moore et al., 1999); DDT induced similarly the inhibition of seed germination in peanut (*Arachis hypogaea*) and mustard (*Brassica juncea*) seeds at concentration of 100 ppm (Mitra and Raghue, 1989). Vidyasagar et al. (2009) studied the importance of endosulfan in mediating stress responses in *Sorghum bicolor*. Their research revealed that endosulfan at different concentrations (0.2, 0.4 and 0.6%) provoked a significant decrease in germination percentage, shoot length, root length and biomass.

Effect of insecticide on seedling development

The results represented in Figures 1 and 2 showed that endosulfan drastically inhibited both shoot and root elongation at any concentration and stage. At concentration of 0.015 g/L, this chemical provoked inhibition of shoot elongation varying between 62% and 75% compared to control (Figure 1). Furthermore, at concentration of 0.06 g/L, which corresponds to field's dose, endosulfan affected strongly the growth process; the inhibition was ranging between 76 and 82% compared to control in the case of shoots.

According to the data presented in Figure 2, we observed that endosulfan affected similarly the root length of the seedlings; the reduction in length of the

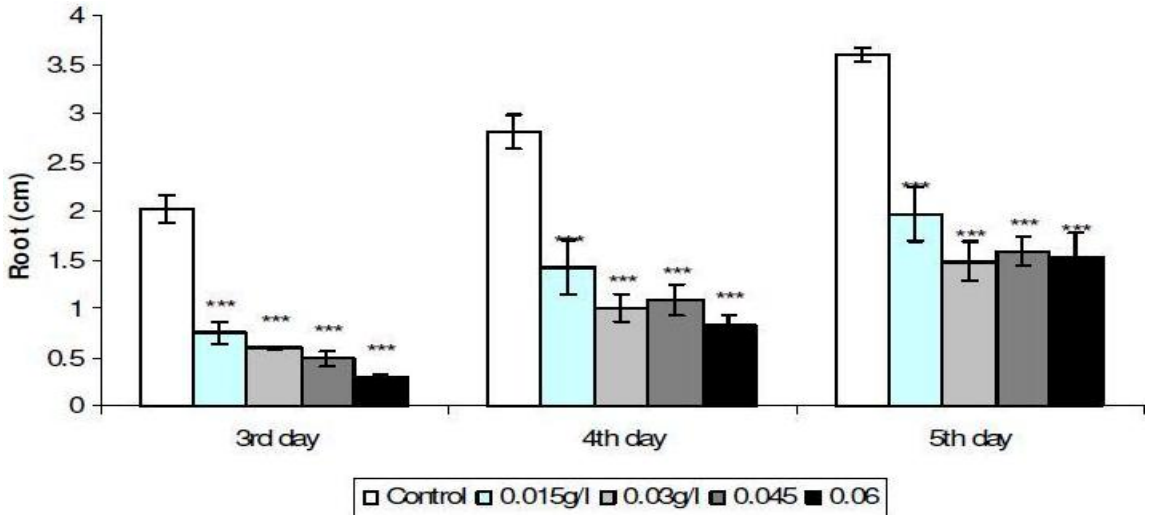

Figure 2. The effect of endosulfan on root length of tomato seedlings. Each value represents the mean + SE of three replications. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively.

root, which was greater in the 3rd day compared to others days of the test, was about 75% compared to control root. Similar to our results are those of Mitra and Raghu (1989) who showed that the growth of *A. hypogaea and B. juncea* seeds was significantly inhibited by the use of DDT. This effect of pesticides on growth was also reported in other studies. Paraquat elicited the effects on both *T. latifolia* root and shoot systems at much lower exposure concentrations (Moore et al., 1999). According to the results of some previous research, a largest decrease of root growth was observed in *Phaseolus vulgaris* and *Pisum sativum* after treatment with chlorsulfuron during the germination process. In both species, root growth was reduced to about 50% compared to control and remained the same with higher chlorsulfuron concentrations (Fayez and Kristen, 1996). Moreover, in the case of *Zea mays* seedlings, it has been observed that primary roots of the seedlings grew significantly slower in the presence of pesticides such as chlorsulfuron and metsulfuron methyl (Fayez et al., 1994).

Pandolfino et al. (1992) explained that the retardation in the growth was caused by destruction of auxins due to the increase in the amount of phenols which was accompanied by the enhancement in the peroxidase activity. Vidyasagar et al. (2009) similarly showed an increase in phenol content in the seeds treated with endosulfan concentrations.

Effect of insecticides on protein contents

Along with changes in germination percentage and root and shoot elongation during the germination process, alterations in protein contents were determined. The results of Figure 3 showed that endosulfan provoked an accumulation in protein contents. In the 3rd day of the test period, proteins accumulation was very important in seeds germinating in presence of endosulfan except at concentration of 0.06 g/l (Figure 3); whereas, in the 4th day, the protein levels were dose dependent. In the 5th day, at field dose, proteins content increased 3, 5 fold compared to control. Proteins are the primary effectors molecules of all living systems; therefore any eventual adaptive response to environmental, physiological or pathological conditions will be translated by alterations in protein activity, location and concentration (Shepard et al., 2000; Bradley et al., 2002).

During the germination process, alterations in protein contents were observed. These results showed that the insecticide used provoked accumulation in protein contents in germinating tomato seeds. In literature, an over expression of proteins of the heat shock proteins 70 (HSP 70) human cell family was extensively used as a marker of an increasing flux of non-native or misfolded proteins resulting from heat shock, oxidative stress, or chemical aggressions (Morimoto et al., 1996; Morimoto, 1998; Croute et al., 2000). Overexpression of HSP 70 was found to occur after exposure to several pesticides including organochlorines (Snyder and Mulder, 2001; Gaubin et al., 2002) and organophosphates (Yang et al., 2002). Moreover, endosulfan, either as pure chemical or as commercial preparation, was found to induce an overexpression of GRP78, the glucose regulated proteins compared to controls. These changes were found to occur at lower concentrations of active molecule (Skandrani et al., 2006). Gianazza et al. (2007) showed that there are obvious differences in protein abundance between protein patterns of whole plantlet extracts from *Lepidium sativum* grown under control conditions and

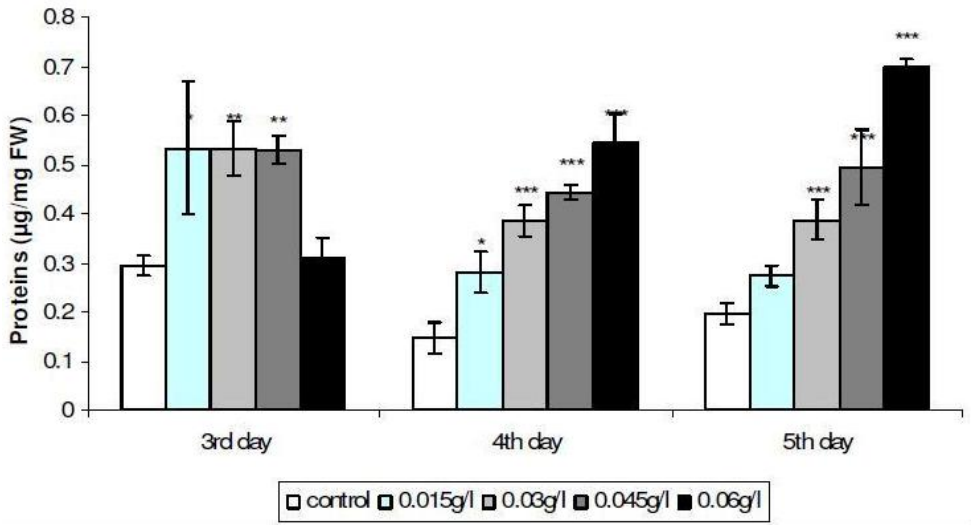

Figure 3. The effect of endosulfan on proteins content in seedlings from germinating tomato seeds. Each value represents the mean + SE of three replications. *, ** and *** indicate significant at *P*≤0.05, 0.01 and 0.001 levels, respectively.

Table 1. The effect of endosulfan on germination percentage of tomato seeds. Each value represents the mean + SE of three replications.

Percentage of germination	Endosulfan				
	0	0.015 g/l	0.03 g/l	0.045 g/l	0.06 g/l
3rd day	97.8	88.4***	89.1***	82.6***	84.05 ***
4th day	95.6	87.85*	86.85***	81.42***	82.85***
5th day	96.42	88.57*	87.14***	82.85***	82.85***

*, ** and *** indicate significant at *P*≤0.05, 0.01 and 0.001 levels, respectively.

Table 2. The effect of endosulfan on proline content in seedlings from germinating tomato seeds. Each value represents the mean + SE of three replications.

Proline µmol/mg FW	Endosulfan				
	0	0.015 g/l	0.03 g/l	0.045 g/l	0.06 g/l
3rd day	266	383.9**	247^{ns}	360.3**	306^{ns}
4th day	281	316.7^{ns}	357.9**	323.2^{ns}	399.2**
5th day	288	397.3**	406.4**	378.3**	291.6^{ns}

*, ** and *** indicate significant at *P*≤0.05, 0.01 and 0.001 levels, respectively; ns as non significant.

after exposure to 200 mg/l cadmium chloride. Bordjiba and Ketif (2009) detected an increase in proteins levels in T*riticum durum* plantlets caused by 3 pesticides treatments which were hexaconazole, bromuconazole and fluazifop-p-butyl.

Effects of insecticides on proline content

Table 2 shows the results obtained from experiments involving proline content in treated and untreated germinating seeds. An increase in proline levels was detected, but the accumulation of this amino acid was more important at the 5th day of test period; it reached 406 µmol/ mg Fw at the concentration of 0.03 g/l. The mechanism of accumulation of proline in plants or plant organs exposed to stress is not well known; it was suggested that it could be due to a decrease in the activity of the electron transport system leading to the accumulation of NaDH and hydrogen ion (H^+) (Venekemp,

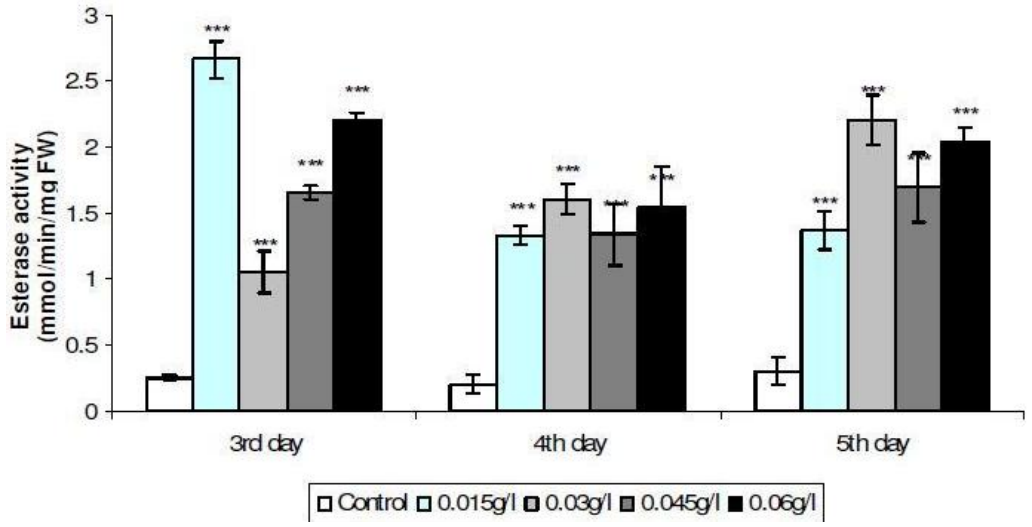

Figure 4. The effect of endosulfan on esterase activity in seedlings from germinating tomato seeds. Each value represents the mean + SE of three replications. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively.

1989; Sawhney et al., 1990; Alia et al., 1993). Proline accumulation (presumably through synthesis from glutamic acid) might be an adaptive mechanism for reducing the level of accumulated NADH and acidity; $2NADH^{+}2H^{+}$ is used to synthesize each molecule of proline from glutamic acid (Venekemp et al., 1987). Concerning the effect induced by pesticides, the proline response is not clear; some related studies showed that there is no effect in proline rate under pesticide stress; for example Norflurazon had no significant influence on proline content of *P. vulgaris,* but was highest in *P. sativum* and *Vicia faba* treated with chlorsulfuron (Fayez and Kristen, 1996).

In our study, the proline level increased in seeds germinating in presence of endosulfan compared to control. Vidyasagar et al (2009) obtained similar results concerning changes in proline content in germinating seeds of Sorghum; they suggested that the increase in this metabolite content was due to endosulfan treatment, which may serve as a mean of protection of plant tissue against chemical stress. Bordjiba and Ketif (2009) detected an increase in proline levels in plants of wheat exposed to 3 different pesticides; they showed that the accumulation of this metabolite is more pronounced in the case of fluazifop-p-butyl and especially at fields dose.

Effects of insecticide on esterase activity

Data of Figure 4 showed a great stimulation of esterase activity in seeds germinating in presence of endosulfan compared to control; this effect was observed concomitantly with time and at all concentrations. Since the 3rd day of the test, endosulfan provoked a strong stimulation in the esterasic activity; for instance, at concentration of 0.015 g/l, the enzymatic activity of esterases reached 2.7 mmol/min/mg FW, whereas in control germinating seeds, it was about 0.25 mmol/min/mg FW. In the 4th and 5th day, the same profile was obtained specially for the concentrations of 0.030, 0.045 and 0.06 g/l. Numerous studies have demonstrated that extensive use of insecticides may result in the development of insecticide resistance and increased esterase activity seems to be a major mechanism of insecticide resistance in many insect species (Devonshire, 1977; Siegfried et al., 1993). In plants, there are few works dealing with the effect of pesticides on esterase activity. Mukherjee et al. (2004) tested esterase activity in exposed and unexposed *Lemna minor* (duckweed) to six heavy metals, and showed that the specific activity of esterase in exposed ones was very higher than the ones of control. In this way, we may speculate that, in tomato seeds, a high increase in general esterasic activity could be explained by an adaptive mechanism to insecticide treatment. Many pesticides and pollutants enter the leaves of plants in the form of hydrophobic esters, which pass readily through the waxy cuticle (Hassall, 1990). Following absorption, ester hydrolysis is a major route of metabolism and this could lead to the inactivation of the xenobiotic (Bounds and Hutson, 2000). While in the abiotic environment, synthetic esters undergo chemical hydrolyses, the available literature would suggest that, in plants, these reactions are catalysed by esterases, as is the case in animals (Hassall, 1990). As an example of bioactivation by hydrolysis in plants is the herbicide

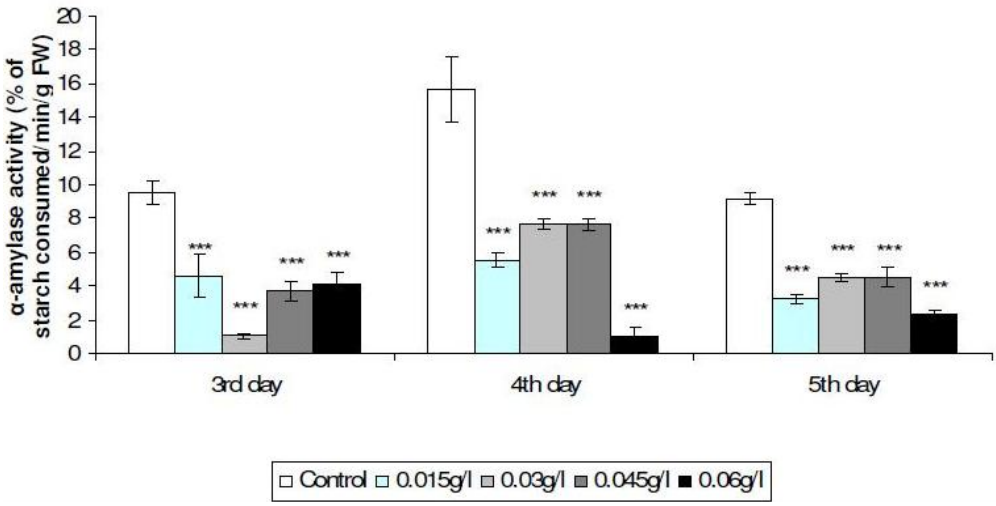

Figure 5. The effect of endosulfan on α-amylase activity in seedlings from germinating tomato seeds. Each value represents the mean + SE of three replications. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively.

thiazopyr which was inactivated by esterase action (Feng et al., 1995).

Effects of insecticides on α-amylase activity

Results of experiments carried out on α-amylase are summarized in Figure 5. A strong inhibition of the enzyme activity in seeds germinating in presence of different concentrations of endosufan was observed. The inhibitory effect was varying between 58.4 and 93.7% compared to control in the 4th day, nevertheless in the 5th day, the inhibition was ranging between 51 and 87.2%. Alpha amylase, enzyme synthesized during germination, is responsible of starch degradation process. In literature, gibberellic acid (GA) is known to induce the synthesis of α-amylase (Chrispeels et al., 1967; Shuster and Grifford, 1962). Mamdouh et al. (1998) suggested that the decrease of α-amylase could result from a loss of endogenous GA. He observed that the reduction in the amount of this phytohormone preceded the decrease of α-amylase activity; this phenomenon confirmed the regulatory role of GA on α-amylase. In this context, Wilkinson (1988) went further in his study to show that alachlor and metolachlor inhibited GA synthesis in sorghum seedlings inducing a decrease in α-amylase synthesis. Similar results were stated by Mamdouh et al. (1998) who showed that metolachlor provoked a significant loss of α-amylase activity in *Z. mays* during germination and growth processes. Alpha amylase activity is also affected by other xenobiotics such as cadmium and copper; Mihoub et al., 2005 reported that this kind of treatment during germination of pea seeds (*P. sativum*) provoked a decline in α-amylase activity; they suggested that this inhibitory effect did not result from starvation in water uptake by seed tissues, but was probably due to a failure in the reserve mobilization process from cotyledons. In contrast, Sabale and Misal (2000) reported a stimulation of α-amylase activity in seeds of jowar germinating in presence of endosulfan and methylparathion.

Effects of insecticides on protease activity

Figure 6 showed a great stimulation of protease activity observed at all concentrations all along the test period. The increase was dose dependant and was 3 fold greater compared to control at fields concentration (0.06 g/l) in both 4th and 5th day.

Rangaswamy et al. (1994) tested the effect of two organophosphorus insecticides, monocrotophos and quinalphos, and two synthetic pyrethroids, cypermethrin and fenvalerate, on protease activity in soil collected from a fallow groundnut field. The results obtained indicated clearly that the insecticides widely used in cultivation of groundnut, at field application rates, enhanced protease activities; the influence of the selected insecticides on such enzyme activities was dose-dependent. Sabale and Misal (2000) showed a varied response of Sorghum bicolor L. seeds under the influence of endosulfan and methylparathion concerning some hydrolytic enzymes during germination. They recorded a stimulation of protease at lower doses of endosulfan (0.05, 0.1% v/v) which was consistent with our experimental results. They also suggested in their comparative study that parathion-methyl imposed a severe osmotic stress during seed germination.

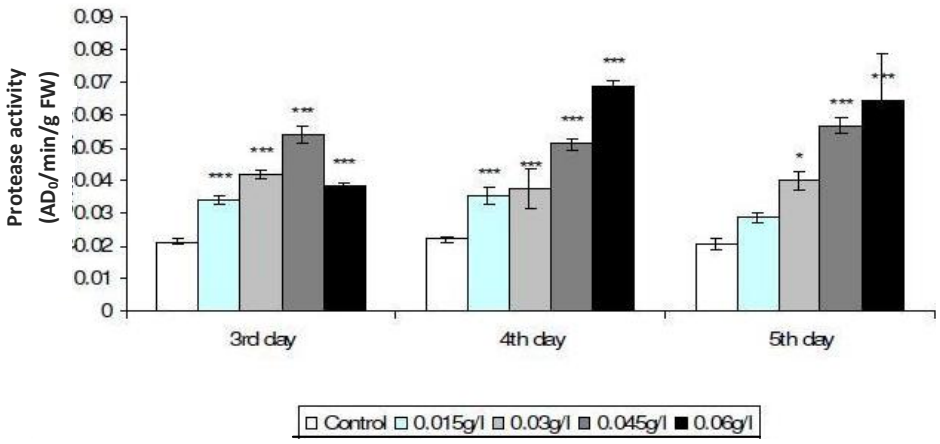

Figure 6. The effect of endosulfan on protease activity in seedlings from germinating tomato seeds. Each value represents the mean + SE of three replications. *, ** and *** indicate significant at $P \leq 0.05$, 0.01 and 0.001 levels, respectively.

Conclusion

To summarize, in the present study, endosulfan showed a profound influence on both root and shoot emergence and post-germinative growth of tomato seedlings; the effects of the insecticide were contrasted in a reduction in seed germination and early seedling growth, which could be attributed to alterations of selection permeability properties of cell membrane and reduction in meristematic cells. On the other hand, we observed an important decrease in α-amylase activity in the treated seeds. Moreover, the increase in esterase activity and the over expression of proteins may lead us to speculate that the plantlets could have developed an adaptive response for the detoxification process. Other experiments undertaken to assess the effect of the same insecticide during late stages of tomato life cycle showed the same profile (data not shown). In order to gain further insight into the physiological changes occurring during insecticides treatment, we are actually conducting a parallel study in order to obtain more indications of the effect of the toxicants on tomato seed germination and the subsequent growth of the seedlings.

ACKNOWLEDGMENTS

This work was supported by the program"CEEM- pole d'excellence regional Regional-AUF project". We are grateful to Mr Nakari abobakr, and Mrs Hajaj Meriem, for their technical help.

REFERENCES

Alia, Pardha Saradhi P, Mohanty P (1993). Proline in relation to free radical production in seedlings of *Brassica juncea* raised under sodium chloride stress. Plant Soil, 155/156: 497- 500.

Aspinall D, Paleg LG (1981). Proline accumulation: physiological aspects. In: Paleg LG, Aspinall D (eds). The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, Sydney, pp. 205-241.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Below FE, Cazetta JO, Seebauer JR (2000). Carbon/nitrogen interactions during ear and kernel development of maize. In: Physiology and Modeling Kernel Set in Maize. Special Publication N° 29: Crop Science Society of America, Madison: WI, pp. 15-24.

Bewley JD, Black M (1985). Seeds: Physiology of Development and Germination. Plenum Press, New York, p 367.

Bordjiba O, Ketif A (2009). Effect of Three Pesticides (Hexaconazole, Bromuconazole and Fluazifop-p butyl) On Some Biochemical Metabolites of the Hard Wheat: *Triticum durum*. Desf. Europ. J. Sci. Res., 260-268

Bounds SVJ, Hutson DH (2000). The comparative metabolism of agrochemicals in plants and animals. In: Roberts T (ed) Metabolism of Agrochemicals in Plants. Wiley, Chichester, pp. 179-210.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254

Bradley BP, Shrader EA, Kimmel DG, Meiller JC (2002). Protein expression signatures: an application of proteomics. Mar. Environ. Res., 54: 373-377.

Chrispeels MJ, Varner JE (1967). Gibberellic acid-induced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiol., 42: 398-402

Croute F, Beau B, Arrabit C, Gaubin Y, Delmas F, Murat JC, Soleilhavoup JP (2000): Pattern of stress protein expression in human lung cell-line A549 after short- or long-term exposure to cadmium. Environ. Health Perspect., 108(1): 55-60.

Cumminsa I, Burnetb M, Edwardsa R (2001). Biochemical characterisation of esterases active in hydrolyzing xenobiotics in wheat and competing weeds. Physiol. Plant., 113: 477-485.

Devonshire AL (1977). The properties of a carboxylesterase from the peach-potato aphid, *Myzus persicae* (Sulz.) and its role in conferring insecticide resistance. Biochem. J., 167: 675-683.

Dwain M (1999). Alfalfa: Seed Germination Seedling Growth Vegetative Development.R-648, (http://www.ag.ndsu.edu/pubs/plantsci/hay/r648w.htm)

Fayez KA, Kristen U (1996). The influence of herbicides on the growth and proline content of primary roots and on the ultrastructure of root caps. Environ. Exp. Bot., 36(1): 71-81.

Fayez KA, Gerken I, Kristen U (1994). Ultrastructural responses of root caps to the herbicides chlorsulfuron and metsulfuron methyl. Plant and

Soil, 167: 127-134.
Feng PCC, Ruff TG, Kosinski WG (1995). Metabolic deactivation of the herbicide thiazopyr by animal liver esterases. Xenobiotica, 25: 27-35.
Gaubin Y, Manceau C, Skandrani D, Croute F, Murat JC, Soleilhavoup JP (2002). Changes in level of genes expression after exposure of cultured human cells to endosulfan as investigated by a cDNA microarrays method. Fresen. Environ. Bull., 8(11): 466-468.
Gianazza E, Wait R, Sozzi A, Regondi S, Saco D, Labra M, Agradi E (2007). Growth and protein profile changes in *Lepidium sativum* L. plantlets exposed to cadmium. Environ. Exp. Bot., 59: 179-187.
Goldraij A, Polacco JC (1999). Arginase is inoperative in developing soybean embryos. Plant Physiol., 119: 297-303.
Goldraij A, Polacco JC (2000). Arginine degradation by arginase in mitochondria of soybean seedlings cotyledons. Planta, 210: 652-658.
Hare PD, Cress WA (1997). Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul., 21: 79-102.
Hassall KA (1990). The Biochemistry and uses of pesticides: structures, metabolism, mode of action and uses in crop protection. 2nd Edition, MacMilan press Ltd, 261 pp.
Kilmer R, Andre AM, Stevens TJ (2001). Pesticide residues and vertical integration in Florida strawberries and tomatoes. Agribusiness, 17: 213-226.
Lea PJ, Joy KW (1983). Amino acids interconversion in germinating seeds. In: Nozillo C, Lea PJ, Loewus FA (eds). Recent Advances in Phytochemistry: Mobilization of Reserves in Germination Volume 17, Plenum Press, New York, pp. 77-109.
Mamdouh M, Nemat A, Nemat MH (1998). Efficacy of exogenous GA, and herbicide safeners in protection of *Zea mays* from metolachlor toxicity. Plant Physiol. Biochem., 36(11): 809-815.
Mihoub A, Chaoui A, El Ferjani E (2005). Changements biochimiques induits par le cadmium et le cuivre au cours de la germination des graines de petit pois (*Pisum sativum L.*). C. R. Biologies, 328 : 33-41.
Mitra J, Raghu K (1998). Effects of DDT on the Growth of Crop Plants. Environ. Pollut., 61: 157-170.
Moore MT, Huggett DB, Huddleston, Rodgers JH, Cooper CM (1999). Herbicide: effects on *Typha Latifolia* (Linnaeus) germination and root and shoot development. Chemosphere, 38 (15): 3637-3647.
Morimoto RI, Kroeger PE, Cotto JJ (1996). The transcriptional regulation of heat shock genes: a plethora of heat shock factors and regulatory conditions. J. Biol. Chem., 77: 139-163.
Morimoto RI (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev., 12(24): 3788-3789.
Mukherjee S, Mukherjee S, Bhattacharyya P, Duttagupta AK (2004). Heavy metal levels and esterase variations between metal-exposed and unexposed duckweed *Lemna minor*: field and laboratory studies. Environ. Inter., 30: 811-814.
Nakashima K, Satoh R, Kiyosue T, Yamagoshi-Shinozaki K, Shinozaki K (1998). A gene encoding proline dehydrogenase is not only induced by proline and hypoosmolarity, but is also developmentally regulated in the reproductive organs of Arabidopsis. Plant Physiol. 118: 1233-1241.
Nanjo T, Kobayashi M, Yoshiba Y, Sanada Y, Wada K, Tsukaya H, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (1999). Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. Plant J. 18: 185-193.
Rangaswamy V, Reddy BR, Venkateswarlu K (1994). Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin and fenvalerate Agriculture. Ecosyst. Environ., 47: 319-326.
Robert H, Giles JR (1980). Wildlife and integrated pest management. Environ. Manage., 4(5): 373-374.
Sabale A, Misal BN (2000). Effect of endosulfan and methylparathion on hydrolytic enzymes in germinating seeds of jowar. J. Environ. Biol., 21(1): 29-35.
Sawhney V, Shearan IS, Singh R (1990). Nitrogen fixation photosynthesis and enzymes of ammonia assimilation and ureide biogenesis in nodules of mungbean (*Vigna radiata*) grown in presence of cadmium. Indian J. Exp. Biol., 28: 883-886.
Shepard JL, Olsson B, Tedengren M, Bradley BP (2000). Specific protein expression signature identified in *Mytilus edulis* exposed to copper, PCB and decrease salinity. Mar. Environ. Res., 50: 337-340.
Shuster L, Grifford RH (1962). Changes in 3-nucleotidase during the germination of wheat embryos. Arch. Biochem. Biophys., 96(3): 534-540.
Siegfried BD, Zera AJ (1994). Partial purification and characterization of a greenbug (Homoptera: Aphididae) esterase associated with resistance to parathion. Pestic. Biochem. Physiol., 49: 132-137.
Skandrani D, Gaubin Y, Vincent C, Beau B, Murat JC, Soleilhavoup JP, Croute F (2006). Relationship between toxicity of selected insecticides and expression of stress protein (HSP, GRP) in cultured human cells: effects of commercial formulations versus pure active molecules. Biochim. Biophys. Acta, 1760(1): 95-103.
Snyder MJ, Mulder EP (2001). Environmental endocrine disruption in decapod crustacean larvae: hormone titers, cytochrome P450, and stress protein responses to heptachlor exposure. Aquat. Toxicol., 55(3-4): 177-190.
Steward N, Martin R, Engasser JM, Goergen JL (1999). Determination of growth and lysis kinetics in plant cell suspension cultures from the measurement of esterase release. Biotech. Bioeng., 66: 114-121.
Strobl S, Maskos K, Wiegand G, Huber R, Gomis-Rüth FX, Glockshuber R (1998). A novel strategy for inhibition of α-amylases: yellow meal worm α-amylase in complex with the *Ragi* bifunctional inhibitor at 2.5 å resolution. Structure, 6: 911-923.
Venekemp JH, Lampe JE, Kout TM (1987). Organic acid as a source of drought-induced proline synthesis in field bean plant *Vicia faba. L.* J. Plant Physiol., 133: 654-659.
Venekemp JH (1989). Regulation of cytosolic acidity in plants under condition of drought. Plant Physiol., 76: 112-117.
Vidyasagar GM, Kotresha D, Sreenivasa N, Karnam R (2009). Role of endosulfan in mediating stress responses in *Sorghum bicolor (L.) Moench.* J. Environ. Biol., 30(2): 217-220.
Walker CH, Mackness MI (1983). Esterases: problems of identification and classification. Biochem. Pharmacol., 32: 3265-3269.
Wilkinson RE (1988). Consequences of metolachlor induced inhibition of gibberellin biosynthesis in sorghum seedlings. Pestic. Biochem. Physiol., 32: 25-37.
Wouters TCAE, Booy G (2000). Stability of esterases and total proteins during seed storage of perennial ryegrass (*Lolium perenne* L.) and their use for cultivar discrimination. Euphytica, 111: 161-168.
Yang D, Lu X, Zhang W, He F (2002). Biochemical changes in primary culture of skeletal muscle cells following dimethoate exposure. Toxicology, 174(2): 79-85.