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Evaluation of the phytotoxicity of a pesticide (TRACTOR 10E) based on Alpha-cypermethrin in two plant species: lentils (*Lens culinaris*) and watercress (*Lepidium sativum*)

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ABSTRACT

Tractor 10E is an Alpha-cypermethrin based insecticide. It is one of the commonly used insecticides. The toxic effect of this product was assessed using the *Lens culinaris* (edible lentil) and *Lepidium sativum* (watercress) test. The seeds of *Lens culinaris* and *Lepidium sativum* were subjected to 6 increasing concentrations of Tractor 10E (25, 50, 100, 200, 400 and 800mg/l) and the control (distilled water). After 7 days, weight, root and stem development were measured. The results of the statistical study revealed the notable effect of this toxic product on growth, especially at high concentrations for the two species which are the subject of plant toxicity tests. Stem length growth is the most sensitive parameter. IC50 is equal to 136.99mg/l for lentil and 136.42mg/l for watercress. The results of this study reveal that this alpha-cypermethrin insecticide has the ability to alter the growth of plants as non-target organisms, which imposes the effective use and management of these toxicants and even replacing them with biopesticides to preserve human health and the environment.

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INTRODUCTION

Intensive agriculture leads to the indiscriminate use of pesticides and fertilizers to optimize agricultural production. In recent decades, the demand, production and use of pesticides and fertilizers continue to increase (PNUE 2022), but in the absence of effective management this has a negative impact on human health and the environment (Yadav et al., 2020; Kumar et al., 2021). In addition, these agrochemicals represent the main pillar of plant protection in the effort to secure food production, with an estimated 20-40% yield loss due to their eventual elimination (Lykogianni et al., 2021).

Pesticide exposure can directly affect all levels of biological organization, including primary producers (Kumar et al., 2021) by altering the pH of the soil and also reducing its fertility (Yadav et al., 2020). This alteration of the soil could lead to a greater or lesser inhibition of

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plant productivity and even a bioaccumulation of contaminants and a transfer to higher levels of the food chain and thus end up in human or animal food. They cause genetic damage, and sperm DNA damage (Alengebawy et al., 2021). Aquatic ecosystems are also negatively affected by pesticide pollution as they move from one ecosystem to another. (Kumar et al., 2021).

In return, sustainable agriculture aims to meet the food needs of a growing world population while ensuring minimal impact on the environment and humans as well as productivity. (Lykogianni et al., 2021).

In Morocco, a survey carried out by Naamane et al. (2020) in a region characterized as a whole by a semi-arid to arid climate points to the extravagant use of many pesticides by farmers to increase yield and alleviate problems associated with crop morphology and growth. This unreasonable use of pesticides therefore constitutes an obstacle to the achievement of sustainable agriculture on a national scale, given their harmful effects on human health and the environment.

Lentil (*Lens culinaris*) and watercress (*Lepidium sativum*) are the most used toxicity test objects, due to their high reactivity to the presence of pollutants and their ability to germinate quickly (Shulaev et al., 2020; Mercado & Caleño, 2021). Therefore, to minimize the harmful effects of agrochemicals on agroecosystems, this study aims to assess the phytotoxicity of an insecticide based on the product Alpha-cypermethrine (TRACTOR 10 E), commonly used in Morocco, Casablanca-Settat region (Naamane et al.,2020) on the growth of stems, roots and fresh weight of two plant species, *Lens culinaris* and *Lepidium sativum*.

MATERIALS AND METHODS

The tested productTRACTOR 10 EC is an insecticide based on 100 g/l of Alpha-cypermethrin from the family of synthetic pyrethroids. This insecticide acts by contact and ingestion on the central and peripheral nervous system of insects in low temperatures as in hot weather, with a pronounced shock action and a long persistence of action allowing it to fight against the main crop pests. It also has repellent properties and inhibits food intake by the larvae (anti-appetence).

The plant material used to assess the impact of the pesticide Tractor, are lentils (*Lens culinaris*) and watercress (*Lepidium sativum*) which are recommended for toxicity testing by the official AFNOR organization (Afnor X31-201, 1982).

The seeds used in the toxicity tests come from the same batch for each plant. They are neither treated with insecticides nor fungicides and they are uniform in size. Two sorting were carried out, the first to keep only the seeds apparently intact (the appearance of the seed coat must not be torn and presenting a homogeneous appearance). The second sorting to eliminate the seeds whose weight is not included in the mean interval 0.0513±0.0046g, corresponding to the mean and the standard deviation calculated from a weighing of 100 seeds taken at random from lentils. Watercress seeds, being very small, are not concerned by the weighing

The final test was preceded by 2 preliminary tests which are the germination test, and the growthtest based on the AFNORX31-201 standard.

The lentil and watercress seeds are placed in 84 glass kneaded boxes (10 seeds per box), whose bottom is covered with a cotton diskette soaked with 10 ml of distilled water for the control, 6 increasing concentrations of a reference product mercury chloride (0.18, 0.37, 0.75, 1.5, 3 and 6mg/l) and Tractor 10E insecticide (25, 50, 100, 200, 400 and 800mg/l) with 3 replicas for the 2 plant species studied.The different concentrations used were based on preliminary studies. All tests and controls were placed in the incubator at a temperature of 20◦C±2 and under illumination of 4300lux ±10% and photoperiod of 16h light/8h dark for 7 days. The weights and the average lengths of the stems and the roots of the seeds having germinated in the control tests and the various concentrations used for the 2 plants species are calculated and are used to express the percentage of inhibition with respect to the control.

The concentrations which caused a 50% inhibition (IC50) of the plant growth of the seeds tested for the various parameters were determined.

Concentrations that caused 50% inhibition (IC50) of plant growth were analyzed by linear regression using the probit method using Microsoft Office Excel 2010 software. Mann Whitney test and Anova test were performed to check if there is a significant difference between the different repetitions and the different concentrations used. Results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

The phytotoxicity of the insecticide was assessed by the percentage inhibition of seedling growth compared to the control.

The results of the phytotoxicity of the insecticide obtained in lentil (*Lens culinaris*) and watercress (*Lepidium sativum*) show that there is no significant statistical difference for the different repetitions carried out (Pvalue>0.05).

The IC50 value of the reference product Mercury Chloride for the duration of the test is 0.75 g/L±2.53 for the lentil and 1.1g/L±3.28 for the watercress. The IC 50 value of this toxic substance in tests for the inhibition of seed germination by a substance should be between 0.5 and 5 g/L according to the recommendations of the AFNORNF X31-201 standard.The Mercury Chloride IC50 values found fall within this range meaning the samples were optimal for testing with the insecticide.

For an exposure period of 7 days, the results reveal the negative impact of the insecticide used (Tractor 10E) compared to the controls (Fig.1 and 2).

The observed growth effects caused by the insecticide increase significantly with increasing concentration used (Figs. 3,4,5,6,7 and 8).The toxic effect of this pollutant on plant growth and condition (plant wilt) was also evident at a concentration above 100mg/L for both species.

in fact, the survey conducted by Naamane et al. (2020) report the problem of extravagant use of insecticides including TRACTOR 10E. Indeed 44% of farmers do not respect the recommended dose (between 100 and 150cc/ha).

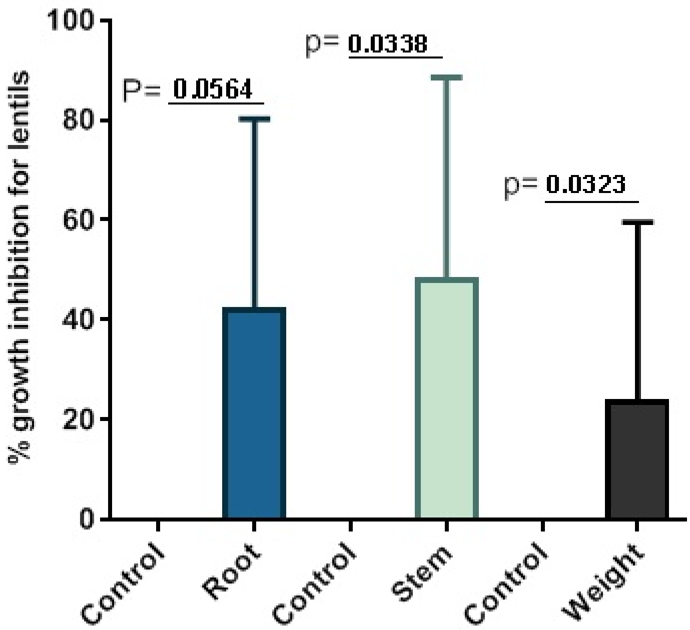

Fig. 1. Percent growth inhibition for lentils

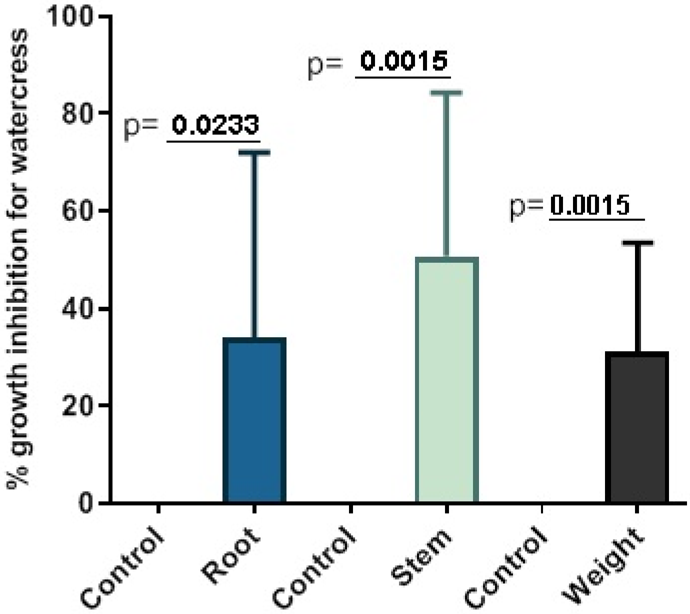

Fig. 2. Percent growth Inhibition for watercress

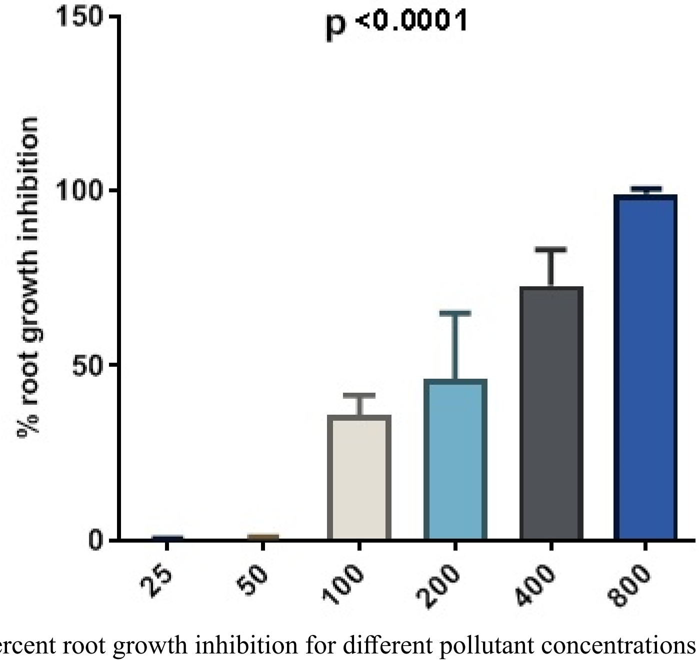

Fig. 3. Percent root growth inhibition for different pollutant concentrations in Lentils

Moreover, pesticide residues have the ability to suppress nitrogen metabolism, increase and decrease the activity of some enzymes (Alengebawy et al., 2021). In addition, leaf pigmentation may be altered and fruits and seeds may stop growing (Alengebawy et al., 2021).Therefore, pesticides can alter plant physiology even if they are not targeted.

For IC50 (Figs. 9, 10), the results of the statistical study reveal a non-significant difference between the different parameters tested for the 2 plants species with respect to the agrochemical product.

In lentils, a concentration of 136.99mg/l ± 14.3 of the insecticide generates 50% inhibition of stem growth and it increases to the concentration of 204.02mg/l ±27.08 for the roots as it passes

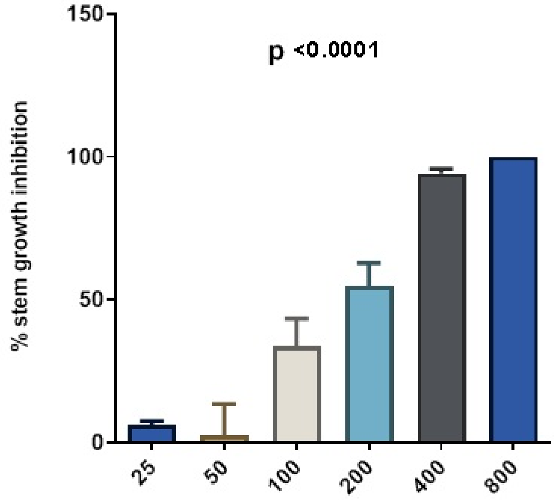

Fig. 4. Percent stem growth inhibition for different pollutant concentrations in lentils

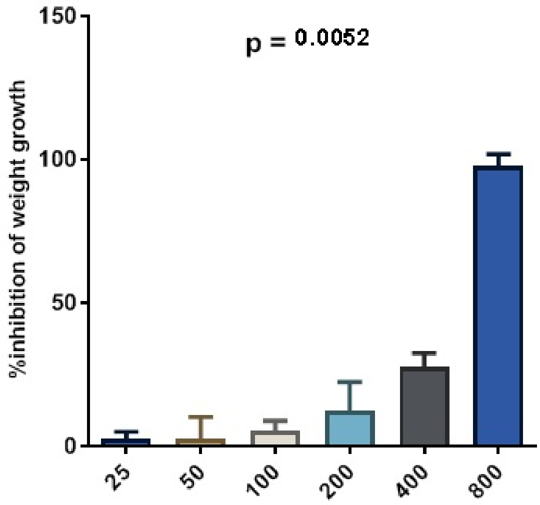

Fig. 5. Percentage inhibition of weight growth for different concentrations of the pollutant in lentils

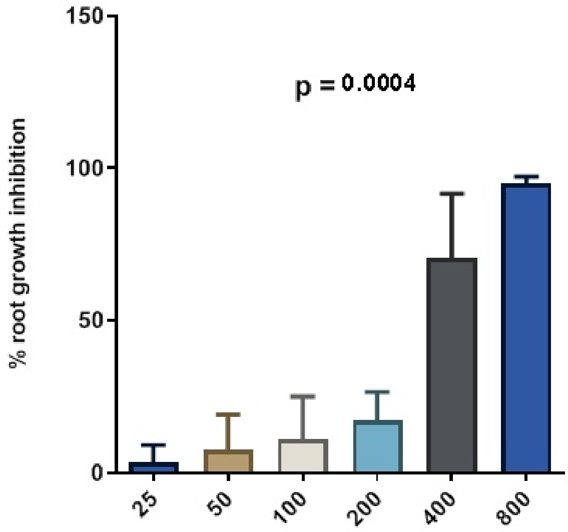

Fig. 6. Percentage inhibition of root growth for different concentrations of the pollutant in watercress.

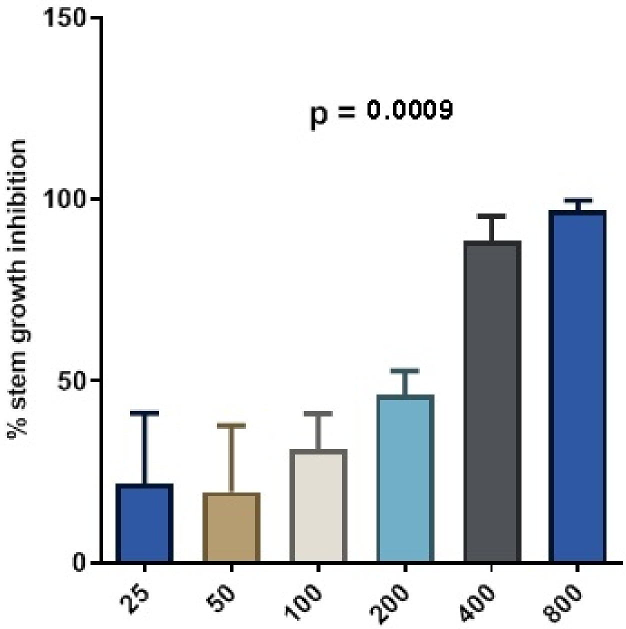

Fig. 7. Percentage of stem growth inhibition for different concentrations of the pollutant in watercress

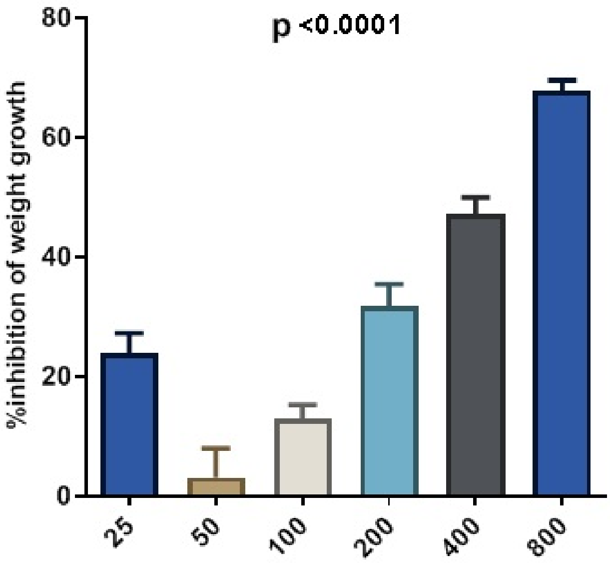

Fig. 8. Percentage inhibition of weight growth for different concentrations of the pollutant in watercress

at a concentration of 666.90 mg/l ±77.87 for the weight.

In watercress, a concentration of 136.42mg/l ± 28.12 of the insecticide generates 50% inhibition of stem growth and it increases to the concentration of 268.96mg/l ±81.30 for the roots as it passes at a concentration of 463.67 mg/l ±12.67 for the weight.

The results reveal that growth inhibition of stem length is the most sensitive parameter against this agrochemical, followed by roots and finally weight.

The results obtained from the phytotoxicity tests showed the possibility of a significant impact of a high concentration of the insecticide on the plants. These data should be of greater concern in taking into account the synergistic effects due to the interaction with other pollutants or in considering the transformation that these chemicals may undergo.

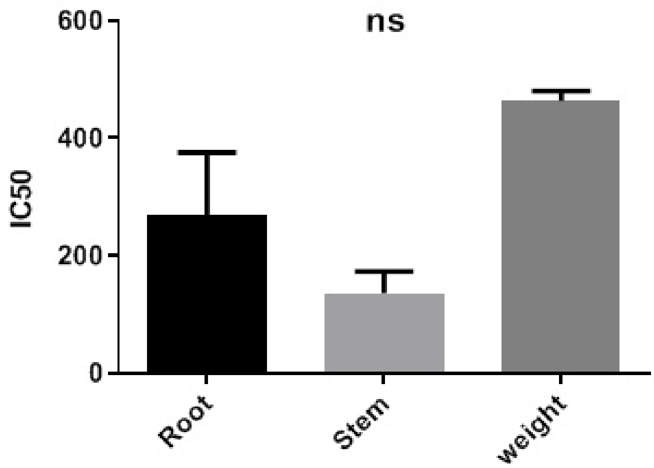

Fig. 9. Estimation of the growth inhibition concentration that affects 50% of the samples (IC 50) in watercress. Ns : no significant difference

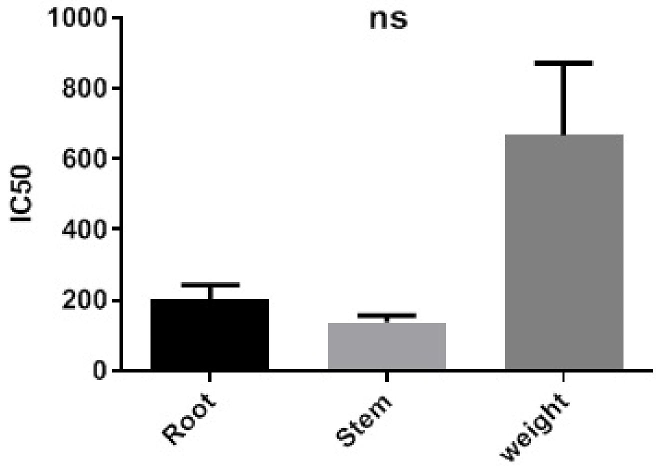

Fig. 10. Estimated growth inhibition concentration affecting 50% of samples (IC 50) in lentils Ns :No significant difference

A similar phenomenon was reported by the study carried out by Piotrowicz-Cieślak et al. (2010). Indeed, exposure of the seeds of 6 legumes: yellow lupine, peas, lentils, soybeans, adzuki beans and alfalfa (3 and 6 days) to sulfamethazine (antibacterial) did not influence germination. Root and stem elongation were more sensitive than germination rate as an indicator of soil contamination by sulfamethazine.

It is found that inappropriate pesticide applications can alter the growth and yield of lentils as well as watercress, with a different sensitivity which may possibly be due to the different physiology of the plant. The studies carried out by Giménez–Moolhuyzen et al. (2020) show that pesticides can produce negative effects on crop physiology, particularly on photosynthesis, leading to a potential decrease in crop growth and yield.

Pesticides and heavy metals top the list of environmental toxins endangering nature (Alengebawy et al., 2021). The extravagant use of pesticides has a negative impact on crops. It affects non-target organisms at different biological scales (Kumar et al., 2021). Exposure to these pesticide residues results in chronic toxicity in living organisms (Kumar et al., 2021) including human (Yadavet al., 2020). They enter the food chain and indirectly cause adverse effects on human health over time (Kumar, et al., 2021).Pesticides have a short-term and long-term impact on health. Residues build up in the body and cause chronic toxicity that leads to serious problems (Yadav et al., 2020). They cause dangerous effects, such as soft tissue sarcoma, ovarian cancer, lung cancer, asthma and endocrine disruption (Alengebawy et al., 2021).

Pesticides play a vital role in reducing disease and increasing crop yields around the world (Tudi et al., 2021). They are responsible for a large part of the pollution of aquatic environments. These chemicals are a stress factor for the aquatic environment which is the final location of microcontaminants (Neury-Ormanni, 2019)and can reach non-target organisms, harming biodiversity (Demarco et al., 2022).The study carried out by Ascoli-Ascoli-Morrete et al. (2022) points to the problem of pesticide bioaccumulation and the higher levels of genetic damage observed in anurans in agricultural areas.This threatens the sustainability of these invertebrates considered as an excellent bioindicator to aquatic ecosystems (Feng et al., 2004).Limb and tail malformations with a significantly higher degree ($p < 0.01$) of nuclear damage in toads (*Sclerophrys regularis*) were observed after exposure to a commonly used pesticide (atrazine) (Said et al., 2022). Anuran tadpoles of *Leptodactylus luctator* and *Physalaemus cuvieri* exposed to imidacloprid insecticide, at environmentally relevant concentrations, show malformations of oral and intestinal structures, reduced body size and activity swimming, micronuclei, and other erythrocyte nuclear abnormalities (Samojeden et al., 2022).

Insecticides have also been shown to adversely affect pollination and natural pest control mechanisms, two important ecosystem services (PNUE, 2022) which compromises the sustainability of agriculture.

Regular and intensive use of pesticides affects cyanobacteria which are very unique photosynthetic prokaryotes with several ecological and economic importance (Tiwari et al., 2019).

In this regard, when pesticides are used to target plants, the behavior of pesticides in the environment, such as transfer and degradation, must be considered (Tudi et al., 2021).

On the other hand, the resistance of pests and vectors to pesticides and their adaptation to the toxins produced by resistant genetically modified crops continues to increase despite great efforts to put in place resistance management strategies worldwide (PNUE, 2022). This evolution has made the management of pests, pathogens and weeds complicated and have pushed up costs (PNUE, 2022). The development of pesticides that have new modes of action capable of rendering resistance ineffective is progressing slowly. This leads to a decrease in crop yields or a drop in the quality of agricultural products (PNUE, 2022).

Pollution by different types of toxic substances, including chemicals applied to kill or contain malignant insects, plant diseases, weeds, gastropods, and other undesirable organisms have adverse effects both on the environment than human (Kumar et al., 2021). They are ubiquitous in the environment and their negative impacts are observed everywhere, in humans, bees, natural enemies of pests, bird populations, aquatic organisms and biodiversity (PNUE, 2022). Indeed, they constitute an obstacle to the achievement of sustainability given their serious threats to human health, agricultural production (plants and soil) and the environment.

To meet the nutritional needs of a growing population, proper use and management of pesticides is essential. For this, a common agricultural policy that better meets societal expectations is more readable and more effective in terms of limiting or even eliminating the use of agrochemicals is necessary. Surveillance methods must be adopted to solve this global health and environment problem.

Moreover, the replacement of chemical control by biological control such as the use of biopesticides should also be developed alongside chemical pesticides to minimize and subsequently eliminate the dependence of agriculture on these toxic chemical compounds. These biopesticides play an important role in the sustainability of the agricultural bioeconomy (Azzaz et al., 2022).

CONCLUSION

At the end of this work, which aims to assess the phytotoxicity of the insecticide: Tractor10E in two plant species: lentil (*Lens culinaris*) and watercress (*Lepidium sativum*), the results and the bibliographic study show that this insecticide based on 'Alpha-cypermethrin has the ability to change the physiology of plants as a non-target organism and therefore alter growth.

Lentil (*Lens culinaris*) and watercress (*Lepidium sativum*) do not show a great difference in sensitivity to the insecticide used, yet the development of the stem remains the most sensitive parameter for watercress and lentils which show a higher sensitivity towards this parameter, followed by root development and finally by weight

This would confirm the inhibitory effect of this agrochemical during unreasonable use and the promotion of sustainable agriculture which is less expensive and with minimal impact on the environment and humans. In this regard, the use of a biopesticide such as *Bacillus thuringiensis* (Microbial Biopesticide) is recommended. This biopesticide has a low persistence in the environment and is specific to the young larval stages of insects (Osman et al., 2015).

Furthermore, the government should take the initiative to prevent the excessive application of these chemicals. This can be done in different ways such as:

- Control of the distribution and use of pesticides and the application of the relevant legislation.
- Continuous training of all stakeholders on the management and effective use of pesticides.
- Reinforcement of standards in this area.
- Prompting of biological control.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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