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Application of poly(epsilon-caprolactone) nanoparticles containing atrazine herbicide as an alternative technique to control weeds and reduce damage to the environment

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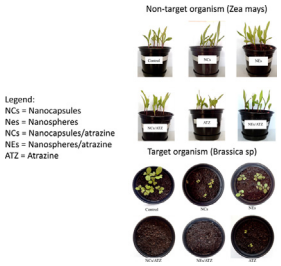
HIGHLIGHTS

- Polymeric nanoparticles change the herbicide release profile.
- PCL nanoparticles used as carrier systems increase herbicide activity.
- Genotoxicity studies showed a decrease in herbicide toxicity after encapsulation in PCL nanoparticles.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanoparticles of poly(epsilon-caprolactone) containing the herbicide atrazine were prepared, characterized, and evaluated in terms of their herbicidal activity and genotoxicity. The stability of the nanoparticles was evaluated over a period of three months, considering the variables: size, polydispersion index, pH, and encapsulation efficiency. Tests on plants were performed with target (*Brassica* sp.) and non-target (*Zea mays*) organisms, and the nanoparticle formulations were shown to be effective for the control of the target species. Experiments using soil columns revealed that the use of nanoparticles reduced the mobility of atrazine in the soil. Application of the *Allium cepa* chromosome aberration assay demonstrated that the nanoparticle systems were able to reduce the genotoxicity of the herbicide. The formulations developed offer a useful means of controlling agricultural weeds, while at the same time reducing the risk of harm to the environment and human health.

1. Introduction

Weeds are ubiquitous in agricultural plantations, where they compete for light and nutrients, hence reducing productivity [1]. Herbicides are therefore widely used to control weeds and promote the preferential growth of the desired species [2].

Herbicides can be classified according to their mode of action, which may be by enzyme inhibition or prevention of cellular growth [3]. Atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine), which is a member of the triazine class of herbicides, is used in many cultivations for pre- and post-emergent control of weeds. Its mechanism of action is by inhibition of photosynthesis [4].

The biological activity of agrochemicals can be reduced by soil sorption and degradation of the active principle. A greater number of applications may therefore be required in order to achieve the

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required results, which can increase the quantities of agrochemical residues in the environment [5].

Bioaccumulation and contamination of the soil with atrazine residues can affect non-target plants in cultivations, reducing productivity [6], while contamination of aquatic systems can have impacts on many species [7–9].

The science of nanotechnology concerns the use of materials at the nanometric scale [10,11]. In agriculture, nanotechnology has been used for the development of sensors to detect diseases, as well as in controlled release systems for nutrients and agrochemicals [12–16].

The advantages of the use of nanoparticles as carriers of active chemicals in agriculture include increased biological activity of the active substances [17,18], reductions in the quantities required [5], and reduced contamination of hydric resources due to leaching [16].

A wide range of different types of particles can be used for this purpose, amongst which are polymeric nanoparticles prepared in the form of nanospheres or nanocapsules. In addition, to their use as carriers for bioactive compounds, these materials are employed to increase the sensitivity of optical diagnosis techniques [19], provide contrast in X-ray analysis [20], assist in corrosion resistance [21], and increase the effectiveness of photodynamic therapy [22].

One of the polymers employed to prepare nanoparticles for use as carriers of biologically active compounds is poly(epsilon-caprolactone), due to its attractive physico–chemical properties, biodegradability, and biocompatibility [23]. The particles produced show good colloidal stability and a high capacity for the encapsulation of bioactive compounds [15,24], making them potentially useful in agricultural applications [25,26].

The objective of the present work was to prepare and characterize polymeric nanoparticles (nanocapsules and nanospheres) composed of poly(epsilon-caprolactone), used to carry the herbicide atrazine, as well as to evaluate the effect of these formulations on target and non-target plants. The migration profile of the active principle through the soil column was investigated, and the genotoxic effects of the formulations were assessed using the *Allium cepa* chromosome aberration assay. The ultimate goal was to develop formulations that cause less damage to the environment and human health.

2. Experimental

2.1. Materials

Atrazine and poly(epsilon-caprolactone) were purchased from Sigma–Aldrich. Acetonitrile (HPLC grade) and methanol (used for the extraction of atrazine from soil) were supplied by J.T. Baker. The solutions were filtered through 0.22 μm filters obtained from Millipore. Plants were grown using a substrate supplied by Orgam Biomix. The chemicals used in the genotoxicity assays (acetic carmine and Schiff's reagent) were purchased from Sigma–Aldrich.

2.2. Preparation of the poly(epsilon-caprolactone) nanocapsules and nanospheres

The nanocapsules and nanospheres were prepared according to the oil-in-water method described by Zhou et al. [27]. The organic phase was prepared using two solutions, the first containing 400 mg of polymer dissolved in 20 mL of dichloromethane, and the second with 10 mg of atrazine dissolved in 10 mL of acetone. The nanocapsules were then produced following addition of 200 mg of Myritol to the solution containing the polymer, while the nanospheres were produced using the polymer solution alone. Subsequent steps were identical for both types of nanoparticle. The solutions were mixed and sonicated in a cold ultrasonic bath for 1 min at 90% power, resulting in formation of the organic phase. This was followed by the addition of 50 mL of a solution of polyvinyl alcohol (3 mg/mL) and cold sonication of the mixture for 8 min at 90% power. The final volume of emulsion (80 mL) was placed in a rotary evaporator for removal of the solvent until the volume was reduced to 10 mL, resulting in a formulation containing 1 mg/mL of atrazine. After preparation, the samples were stored in amber flasks at ambient temperature.

2.2.1. Physicochemical stability

The physicochemical stability of the nanoparticles was evaluated using measurements of size, polydispersion index, zeta potential, pH, and encapsulation efficiency, conducted over a period of 90 days. The samples were analyzed in triplicate at 25 °C.

2.2.2. Size and polydispersion index

The dynamic light-scattering (photon correlation) technique was used to measure the size of the nanoparticles. The colloidal emulsions were diluted in deionized water (1:1000, v/v) and analyzed in triplicate, at 25 °C, using a Zetasizer Nano ZS90 (Malvern Instruments, UK) with a fixed angle of 90°.

2.2.3. Zeta potential

The zeta potential (expressed in mV) describes the surface charge of the nanoparticles, with higher values indicating greater particle stability [28]. The colloidal emulsions were diluted in deionized water (1:1000, v/v) and analyzed in triplicate, at 25 °C, using the Zetasizer instrument, as described above.

2.2.4. Encapsulation efficiency

The encapsulation efficiency was determined in two steps. Firstly, 100 μL of the nanocapsules or nanospheres was dissolved in 900 μL of acetonitrile to obtain a solution containing 100% of the atrazine active principle, which was then filtered through a 0.22 μm membrane (Millipore). The second step was to filter 400 μL of the nanocapsules or nanospheres through a Microcon ultrafiltration unit (Millipore) composed of regenerated cellulose with an exclusion pore size of 30 kDa. The system was centrifuged at 200 × *g* for 30 min, after which 100 μL of the filtrate containing free atrazine was diluted in 900 μL of deionized water. The samples were analyzed by high performance liquid chromatography (HPLC), using a Varian ProStar instrument equipped with a PS-210 pump, an OS 325 UV–vis detector, a META THERM oven, and an autosampler. The chromatograms were analyzed using Galaxy Workstation software. The mobile phase consisted of acetonitrile/water (50:50, v/v) at a flow rate of 1 mL/min, and the detector wavelength was set at 220 nm. These conditions were used to generate a calibration curve ($r = 0.9993$), and the limits of detection and quantification were 0.57 and 1.91 μg/mL, respectively. The same quantification conditions were used throughout this work.

2.3. Release kinetics assays

The release kinetics experiments were performed using a system consisting of donor and acceptor compartments. The donor compartment contained 2 mL of the nanocapsule or nanosphere emulsion containing atrazine (or a solution of free atrazine), and was separated from the acceptor compartment by a 1 kDa molecular exclusion pore size cellulose membrane (Spectrapore). The acceptor compartment contained 250 mL of deionized water maintained under constant agitation using a magnetic stirrer. Samples (1 mL) were periodically collected from the acceptor compartment for subsequent analysis by HPLC, and 1 mL of deionized water was added to the system after each collection. The samples were