Humic substances-based nanoparticles as a novel nano-delivery system for an RNAi-mediated control strategy against *Spodoptera littoralis*

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To limit the use of chemical insecticides and develop bioinspired control strategies for phytophagous insects, some bioactive molecules have been already identified by exploiting insect-natural antagonist interactions. These include a target-specific dsRNA that, when orally delivered, is able to silence the immune gene *Sl102* (ds*Sl102*) in *Spodoptera littoralis* larvae, inducing immunosuppression and increasing the insect susceptibility to *Bacillus thuringiensis* toxins. Due to its extreme sensitivity to environmental degradation, ds*Sl102* must be protected by sustainable formulations when sprayed in the field. These formulations must have specific characteristics, such as (i) preserving the integrity of ds*Sl102* and increasing shelf-life and storage stability; (ii) enabling rapid and efficient penetration of ds*Sl102* within plant tissues, which are ingested by the chewing insect; (iii) ensuring the release of ds*Sl102* inside the insect's gut lumen.
In this context, the use of sustainable materials to produce nano-delivery systems can be potentially beneficial. Three formulations composed of plant-based materials and with different physical and chemical characteristics are presently under development and analysis.
Here we report the use of new nanocarriers based on humic substances derived from the degradation of vegetable and coffee husks, conjugated with chitosan (HS-Chi NPs). These NPs can encapsulate ds*Sl102* with an efficiency of over 90% and preserve ds*Sl102* from environmental degradation for up to 12 months. To study their fate within plants, we applied fluorescent-labelled HS-Chi NPs to tomato plants *via* foliar spraying. All the administrated HS-Chi NPs penetrate the cuticle of leaves within 3 hours and remain stable without degradation in the apoplast of the upper epidermal layer for at least 96 hours. Notably, the HS-Chi NPs did not alter the photosynthetic efficiency of treated plants indicating their compatibility with plant physiology. In addition, while 3rd instar *S. littoralis* larvae fed with tomato leaves sprayed with naked ds*Sl102* did not show any gene silencing in the haemocytes, those fed with leaves treated with ds*Sl102* encapsulated within HS-Chi NPs showed a significant reduction in *Sl102* gene expression, demonstrating the RNAi efficacy of the HS-Chi NPs-*dsSl102* complex. Overall, our results strongly suggest the potential use of HS-Chi NPs as efficient biocompatible carrier for the delivery and protection of silencing dsRNA molecules in the field. The proposed nano-delivery strategy might be profitably used also for other bioactive molecules, such as peptides and proteins.