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Short Communication

Enhancing Actara and Bayleton Detoxification in Greenhouse Cucumbers Using Plant Growth Stimulants

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Crops cultivated in protected environments often require pesticide applications, which can increase the risk of pesticide residues due to short harvest cycles and slow detoxification. This study tested the efficacy of Biohumus and Fertimicro for accelerating the dissipation of two commonly used pesticides, Actara (thiamethoxam) and Bayleton (triadimefon), in greenhouse-grown cucumbers. Biohumus, an organic amendment rich in beneficial microbes and humic substances, and Fertimicro, a microfertiliser containing essential nutrients, were applied alongside pesticides. Residue levels were analysed 1, 3 and 5 days after treatment. Both biostimulants significantly enhanced pesticide degradation compared to untreated controls. The study revealed significant pesticide residues in cucumber fruits one day after treatment. In the days following spraying, intensive detoxification of pesticide residues occurred, leading to a significant decline in Bayleton and Actara residue levels. Stimulant-based solutions significantly enhanced pesticide detoxification compared to water-based treatments. Without biostimulants, Actara residues in cucumber fruits exceeded the MRL (maximum residue limit) fourfold, even three days after spraying (PHI – pre-harvest interval). Biostimulant application reduced Actara residues to 0.08 ± 0.01 mg/kg with Biohumus and within the limit with Fertimicro (0.05 mg/kg, $p = 0.0044$). A similar trend was observed for Bayleton: by day 5 (PHI), Biohumus- and Fertimicro-treated fruits met the MRL (0.5 mg/kg), whereas untreated fruits exceeded the limit by 1.6 times ($p = 0.00051$).

Keywords: pesticides, Biohumus, Fertimicro, protected cultivation, pre-harvest interval, maximum residue limits

Crops cultivated in protected environments, such as greenhouses, often require frequent pesticide applications to manage pests and diseases. Due to the short harvest cycles of many greenhouse crops, including cucumbers and tomatoes, pesticide residues may persist beyond the designated pre-harvest interval (PHI), at times exceeding maximum residue limits (MRLs). Environmental factors – such as temperature, humidity, and ventilation – further influence pesticide degradation rates, underscoring the need for effective strategies to accelerate detoxification in produce destined for consumption.

Recent research suggests that plant biostimulants and plant growth regulators (PGRs) can enhance pesticide detoxification while increasing crop resilience to environmental stress. These substances mitigate the phytotoxic effects of pesticides and may function as safeners by limiting absorption and toxicity. Furthermore, they have been shown to stimulate enzymatic detoxification pathways, activate plant defence responses, and support microbial communities involved in pesticide degradation (Ma *et al.* 2018; Liu *et al.* 2021; Kumari *et al.* 2023; Katsenios *et al.* 2023; Banu *et al.* 2024).

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In this study, we assessed the potential of two biostimulants – Biohumus and Fertimicro – to accelerate pesticide detoxification in greenhouse-grown cucumbers. Focus was placed on two commonly used agrochemicals: the insecticide Actara (thiamethoxam) and the fungicide Bayleton (triadimefon).

Biohumus, also known as vermicompost, is an organic fertiliser rich in nutrients, enzymes, phytohormones, humic substances, amino acids, natural antibiotics, and beneficial microorganisms. These include nitrogen-fixing bacteria (e.g., *Rhizobium*, *Azotobacter*), phosphate-solubilising microbes (*Penicillium*, *Bacillus*), decomposers, and various fungi and yeasts with plant growth-promoting properties.

Fertimicro, on the other hand, is a mineral-based microfertiliser designed for foliar applications. It contains a balanced mixture of macronutrients (N, K, Mg) and a high concentration of essential micronutrients, including boron, copper, iron, manganese, zinc, and molybdenum, many of which are chelated to improve absorption.

The experiment was conducted on the "Gold" variety of Dutch "Gold Wagner F" hybrid cucumbers grown in a greenhouse in Armenia. Cucumber plants were sprayed with Actara and Bayleton at a rate of 0.6 kg/ha each. Biohumus and Fertimicro, diluted at ratios of 1:10 and 1:100 respectively, were used as diluents for pesticide applications, while ordinary water was used in the control treatment.

Spraying was conducted once during the mass ripening stage. Cucumber fruits were sampled on days 1, 3 and 5 after application to assess residue levels.

Pesticide residues were detected using thin-layer chromatography (Unified Method… 1992; Avetisian 2005). Samples were extracted with 50% aqueous acetone and re-dissolved in chloroform. For Actara, the mobile phase consisted of acetone and n-hexane (1:1), with detection via an ammonia solution of silver nitrate and UV exposure. For Bayleton, the mobile phase was n-hexane and acetone (4:1), and detection involved bromophenol blue and $AgNO_3$. Thin-layer silica gel plates ("Silufol UV 254", Czech Republic) were used as the stationary phase.

Data were analysed using ANOVA at a 95% confidence level, based on four replicates. Results are presented as means ± standard deviation (*SD*). Significant differences among treatments were determined using the least significant difference (*LSD*) test and Tukey's significant difference (*HSD* – honestly significant difference) test.

The study revealed significant pesticide residues in cucumber fruits one day after treatment (Table 1). Actara levels ranged from 0.7±0.15 to 1.2±0.01 mg/kg, and Bayleton from 2.6±0.18 to 3.2±0.22 mg/kg. These high values likely reflect the pesticides' ability to penetrate the cuticle and translocate into the fruit with nutrients from leaves and stems. Once inside, plant detoxification processes began reducing their toxicity.

In the days following application, intensive detoxification of pesticide residues occurred in the fruits, leading to a significant decline in both Bayleton and Actara levels. The application of biostimulants enhanced this effect, accelerating pesticide detoxification in cucumber fruits. However, detoxification rates varied between the two pesticides and depended on the biostimulant used.

One day after spraying, total residues in Biohumus-treated variants were 25% lower for Actara and 41.7% lower for Bayleton compared with the control ($p = 0.0079$). Within three days, Actara residues declined 60% faster with Biohumus and 75% faster with Fertimicro compared to the control. Similarly, Bayleton residues decreased by 28.6% with Biohumus and 42.9% with Fertimicro ($p = 0.0053$). This trend persisted over five days, demonstrating sustained effects of the biostimulants. *LSD* values confirmed that reductions exceeding these thresholds were statistically significant (see Table 1).

Although the results indicate rapid detoxification of Actara and Bayleton in biostimulant-treated fruits, it remains crucial to assess whether this ensures a safe harvest within the defined pre-harvest intervals (PHIs).

Residue levels were compared with the maximum residue limits (MRLs) set by the European Commission (EC, 2005) and the Customs Union (CU, 2010). For cucumbers grown under protected conditions, the MRLs are 0.05 mg/kg for thiamethoxam (Actara) and 0.5 mg/kg for triadimefon (Bayleton). The defined PHIs are three days for Actara and five days for Bayleton (HS-2.3539-18 (2019)).

In untreated cucumbers (Control), Actara residues at the PHI exceeded the MRL by a factor of four (see Figure 1). Biostimulant treatments accelerated detoxification, reducing Actara levels to 0.08 ± 0.01 mg/kg with Biohumus, and to within the legal limit with Fertimicro (0.05 ± 0.01 mg/kg, p = 0.0044). A similar trend was observed for Bayleton: at the PHI, fruits treated with Biohumus and Fertimicro complied with the MRL, whereas residues in untreated fruits exceeded the limit by 1.6 times (p = 0.00051).

Tukey's *HSD* test confirmed statistically significant differences ($p < 0.05$) between the Control and biostimulant-treated groups.

These findings are consistent with previous research demonstrating the role of humic substances in pesticide degradation. Studies conducted in Turkey showed that imidacloprid degrades more rapidly in the presence of humic acid (Yılmaz *et al.* 2012). Similarly, research by Ćwieląg-Piasecka *et al.* (2018) and Alister *et al.* (2020) indicates that humic acid can reduce pesticide usage by 20 – 30%, highlighting its potential as an effective detoxification agent.

Biohumus contains a rich mix of microbes, enzymes, yeasts, and fertility-enhancing microorganisms that support plant growth and aid in pesticide metabolism, thereby reducing toxicity. Banu *et al.* (2024) highlighted the importance of microbial adaptation, synergistic consortia and horizontal gene transfer in pesticide degradation. For example, *Bacillus* and *Pseudomonas* species cooperate to break down organophosphate pesticides via hydrolysis and oxidation. These microbes continuously adapt, modifying metabolic pathways to utilise pesticides as energy sources. Furthermore, bioactive compounds in Biohumus – such as amino acids, polysaccharides, vitamins, and essential nutrients – stimulate plant metabolism, enhancing resilience and contributing to a more sustainable detoxification process (Besaliev *et al.* 2021).

While the pesticide-degrading properties of Biohumus and humic acids are well documented, Fertimicro's specific effects on pesticide degradation remain relatively unexplored.

The enhanced detoxification of pesticide residues observed in cucumber fruits is likely attributable to the activation of physiological and biochemical processes within the plants. Despite structural differences among pesticide molecules, they typically undergo similar plant detoxification pathways, which include enzymatic transformation, conjugation, sequestration, and the activation of stress responses. When pesticides accumulate at high concentrations, plants initiate defence mechanisms, including the production of enzymes to neutralise harmful compounds. In the initial phase, key enzymes such as cytochrome P450s, esterases and hydrolases catalyse oxidation, reduction, and hydrolysis reactions. These are followed by conjugation processes, where the modified pesticide molecules are bound to glutathione, sugars, or amino acids, increasing their solubility for transport and storage (Van Ferd *et al.* 2003).

T a b l e 1

Detoxification of Actara and Bayleton in cucumber following application with biostimulants [mg/kg]

Pesticide	Biostimulant	Days after the application		
		1	3	5
LSD		0.26	0.08	0.03
Actara	Control	1.2 ± 0.08	0.2 ± 0.08	0.07 ± 0.03
	Biohumus	0.9 ± 0.22	0.08 ± 0.01	0.04 ± 0.02
	Fertimicro	0.7 ± 0.15	0.05 ± 0.01	0.02 ± 0.01
LSD		0.31	0.24	0.21
Bayleton	Control	3.2 ± 0.22	1.4 ± 0.17	0.8 ± 0.14
	Biohumus	2.8 ± 0.18	1.0 ± 0.17	0.4 ± 0.15
	Fertimicro	2.6 ± 0.18	0.8 ± 0.08	0.2 ± 0.10

Note: *LSD* – least significant difference.

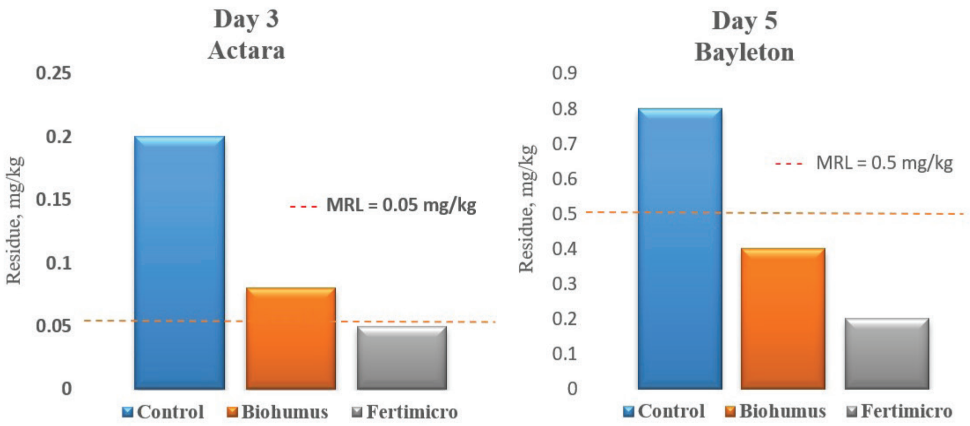

Figure 1. Comparison of residue levels of Actara (Day 3) and Bayleton (Day 5) in cucumber under Control, Fertimicro, and Biohumus treatments, relative to their respective MRLs (red dashed line: Actara – 0.05 mg/kg; Bayleton – 0.5 mg/kg).
Note: MRLs – maximum residue limits.

To combat oxidative stress induced by pesticide exposure, plants also activate antioxidant enzymes – including superoxide dismutase (SOD), catalase (CAT) and various peroxidases – which help neutralise reactive oxygen species (ROS) (Hasanuzzaman *et al.* 2020; Zhang *et al.* 2024). This upregulated metabolic activity facilitates the breakdown of complex pesticides into simpler, non-toxic compounds, which are ultimately removed from plant tissues through physiological processes such as transpiration, guttation, and exudation.

Fertimicro's rich micronutrient composition suggests it may support plant detoxification mechanisms by enhancing enzymatic activity, promoting conjugation processes, and strengthening stress responses. These functions may complement the microbial degradation and organic matter interactions facilitated by Biohumus. Micronutrients such as zinc (Zn), manganese (Mn), and cobalt (Co) are known to activate antioxidant enzymes that mitigate oxidative stress. Furthermore, iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) serve as cofactors for cytochrome P450 enzymes, which are central to the oxidative transformation of pesticide molecules. Sulphur (S) and magnesium (Mg) contribute to glutathione synthesis, further supporting detoxification through conjugation (Johnson & Mirza 2020; Kumar *et al.* 2021).

In conclusion, biostimulants such as Biohumus and Fertimicro significantly accelerated the detoxification of Actara and Bayleton residues in greenhouse-grown cucumbers. Without biostimulants, pesticide levels exceeded safe limits at the pre-harvest interval, while treated fruits remained within or below maximum residue limits. This highlights the dual benefit of biostimulants in enhancing food safety and promoting plant health. Their use offers a promising, sustainable strategy for reducing chemical residues in crop production. To fully understand the role of biostimulants in pesticide detoxification, future research should explore key biological markers – such as enzyme activity, gene expression, metabolomic changes, and physiological responses. This integrated approach will deepen our understanding of plant resilience and support the development of cleaner, safer agricultural practices.

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