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Neem Oil and *Bacillus thuringiensis* as Eco-Friendly Alternatives to Synthetic Pesticides in Maize Fields

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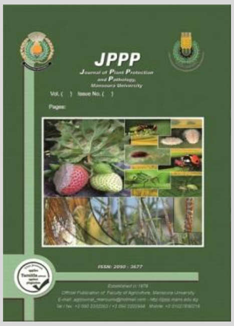

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ABSTRACT

The present study conducted to assess the residue levels chlorpyrifos, imidacloprid, lambada-cyhalothrin, oil neem and Bacillus thuringiensis in maize. In addition, tested the effect their effect on plant growth, and antioxidant responses. The residue monitoring showed that chlorpyrifos and imidacloprid persisted the longest period after application, with half-lives of 28.2 and 22.7 days ،respectively, and both exceeded maximum residue limits. Lambda-cyhalothrin (17.9days) stayed within safe levels, while no residues were detected for neem oil or Bacillus thuringiensis (Bt), highlighting their environmental safety. Plant growth clearly reflected these differences. Chlorpyrifos reduced plant height to 189.2 cm compared with the control (218.3 cm) and yield to 7.42 t/ha (a 24.5% drop), followed by imidacloprid (195.6 cm; 7.85 t/ha; 20.1% loss respectively) and lambda-cyhalothrin (205.3 cm; 8.42 t/ha; 14.3% loss respectively). In contrast, neem oil (214.9 cm; 9.48 t/ha; 3.6% loss) and Bt(216.4 cm; 9.64 t/ha; only 1.9% loss) compare to the growth and yield close in the untreated control. The antioxidant profile took the same pattern where, Chlorpyrifos and imidacloprid triggered oxidative stress, lowering DPPH scavenging activity (42.0–44.3%) and phenolic content (1.82–1.91 mg GAE/g), while raising malondialdehyde (12.8–14.2 nmol/g). Neem oil and Bt had the opposite effect, boosting antioxidant activity (67.5% and 63.2%) and phenolics (3.03 and 2.89 mg GAE/g), while keeping lipid peroxidation much lower (6.9–7.2nmol/g). Overall, synthetic pesticides resulted in high residues compared to bio insecticides. Moreover, synthetic pesticides stressed the plants while the tested biopesticides proved safer, supporting both plant health and antioxidant defenses.

Keywords: synthetic pesticides, biopesticides, pesticide residue

INTRODUCTION

Pesticides are extensively used in agriculture to control pests and disease vectors, thereby improving food production. Globally, about 4.6 million tons of pesticides are applied annually (Zhang *et al.,* 2011). However, their extensive use poses significant risks as major environmental contaminants,, they can leave toxic residues that contaminate soil, water, air, and food, negatively impact non-target organisms, endanger human health, and cause economic damage (Hashmi *et al.,* 2011; Ahmed *et al*., 2011; Anwar *et al.,* 2012). The misuse of pesticides can contribute to the development of various human illnesses. Contact with these chemicals has been linked to a wide range of health problems, such as nausea, headaches, hormonal disturbances, and even cancer (Yousefi *et al*., 2022).

Agrochemicals, such as pesticides and fertilizers, are commonly applied to protect grains from pests and to enhance crop yields per unit area. Due to their chemical characteristics, these substances can migrate into grains (Dors *et al.,* 2011). Among these, organophosphate pesticides (OPs), a widely used class of insecticides, act by inhibiting acetylcholinesterase activity in insects' nervous systems. Chlorpyrifos is considered moderately toxic and primarily affects both the central and peripheral nervous systems by blocking the activity of the enzyme acetylcholinesterase (Smegal, 2000; Phung *et al.,* 2012). Pyrethroids, on the other hand, are synthetic organic chemicals, and exposure to them may trigger a wide range of health issues such as seizures, dizziness, nausea, headaches, mouth ulcers, vomiting, chest tightness, sore throat, excessive secretions, heart palpitations, blurred vision, abdominal pain, and in severe cases, even coma (Bradberry *et al.,* 2005). Lambda-cyhalothrin , a Type II synthetic pyrethroid, is among the most commonly used pesticides. Due to its high efficacy and toxicity profile, it is often preferred over organochlorines, organophosphates, and carbamates (Pauluhn, 1999). This insecticide is extensively applied in cotton cultivation, vegetable production, and for the management of a broad spectrum of insect pests across different crops (Schenone & Rojas, 1992; Awumbila & Bokuma, 1994). Imidacloprid, a neonicotinoid insecticide patented in 1985 (Tomizawa & Casida, 2005), is currently registered in more than 120 countries for use on over 140 crops (Jeschke *et al*., 2011). It exhibits systemic activity, particularly effective against piercing-sucking insects, and has also been successfully applied in controlling fleas in cats and dogs (Tomizawa & Casida, 2005). The mode of action involves disrupting the nicotinic neuronal pathway in the insect nervous system, leading to overstimulation, paralysis, and ultimately death at effective concentrations (Chen *et al*., 2014; Jeschke *et al*., 2011; Tomizawa and Casida, 2005). Neem oil, extracted from the Neem tree (Azadirachta indica Juss), is widely recognized for its phytochemicals used in health and pest control (Norten and Pütz, 1999; Forim *et al*., 2014). It contains over 100 active compounds, mainly limonoids, with azadirachtin responsible for about 90% of its insecticidal action by disrupting microtubule formation (Morgan, 2009). Compared with synthetic pesticides, neem oil is safer for the environment and non-target organisms due

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to its selectivity and rapid degradation (Koul *et al*., 1990 and Quarles, 1994).

Bacillus thuringiensis (Bt) has long been applied in agriculture due to its insecticidal proteins, making it an eco-friendly biopesticide. Its insecticidal effect mainly comes from δ-endotoxins, particularly cry proteins, along with other vegetative and secreted proteins that act against specific insect species Kumar *et al*., (2021).

Pre-harvest intervals (PHIs) play a critical role in field-based residue studies. They represent the period between pesticide application and crop harvest, and strict adherence to these intervals is essential to ensure that the produce meets food safety standards and remains within legal residue limits Duman and Tiryaki (2022).

In light of these issues, the present study focuses on assessing the residual levels of selected pesticides and their impact on maize plants. Specifically, the current work investigates the effect of the tested pesticides on plant growth parameters as well as the antioxidant defense system. By comparing the effects of synthetic pesticides with biopesticides such as neem oil and *Bacillus thuringiensis* (Bt), the study aims to identify safer and more sustainable alternatives that minimize residue accumulation while supporting plant health and stress tolerance.

MATERIALS AND METHODS

Experimental Site Characterization

Field trial was conducted during the 2023 growing season (May–September) at the Giza Agricultural Research Station, Egypt (30°01′15″N 31°13′38″E); with 28.3°C mean temperature and 18.7 mm monthly precipitation. The type of the soil was sandy-loam soil, with 72% sand, 18% silt, and 10% clay, pH around 7.23, and 1.21% organic matter.

The Experimental Design

The experiment followed a randomized complete block design (RCBD) with five treatments and untreated plot with four replicates. Each plot measured 6 × 5 m (30 m²) and was separated by 1.5 m borders. Maize hybrid Pioneer P1234 was planted at a density of 60,000 plants/ha with 70 cm spacing between rows. Good agricultural practice was applied, including basal fertilization (120 kg N/ha, 60 kg P_2O_5/ha, 80 kg K_2O/ha) and drip irrigation to maintain 70% field capacity.

Table 1. Treatment Specifications

Treatment	Formulation	Active Ingredient Concentration	Application Rate	ADImg/kg bw/day (EPA)
Chlorpyrifos	EC 20%	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate	1.5 L/ha	0.01
Imidacloprid	SC 20%	1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	0.5 L/ha	0.02
Lambda-cyhalothrin	CS 5%	[1α(S),3α(Z)]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate	0.2 L/ha	0.04
Neem oil	Cold-pressed EO	Azadirachtin (3,000 ppm)	3.0 L/ha	No risk
B. thuringiensis	WP	Cry1Ab δ-endotoxin (8 × 10^4 CFU/g)	2.0 kg/ha	No risk
Control	-	-	-	-

All treatments were applied at V6 growth stage using calibrated knapsack sprayers delivering 500 L/ha with 0.1% v/v non-ionic surfactant .

Determination of pesticides residue in maize plant:

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was applied for the determination of pesticide residues in maize samples (leaves). Approximately 10 g of homogenized maize sample was placed into a 50 mL centrifuge tube, followed by the addition of 10 mL acetonitrile (MeCN). The mixture was vortexed for 1 min to extract pesticide residues. A salt mixture of 4 g anhydrous magnesium sulfate ($MgSO_4$) and 1 g sodium chloride (NaCl) was then added to the sample and the solvent. After vigorous shaking for 1 min, the tubes were centrifuged at 4,000 rpm for 5 min to allow phase separation. After that, a 6 mL aliquot of the supernatant was transferred into a 15 mL centrifuge tube containing 150 mg primary secondary amine (PSA), 150 mg C18, and 900 mg $MgSO_4$. The tube was vortexed for 1 min and centrifuged under the same conditions. The cleaned extract was filtered through a 0.22 µm PTFE syringe filter before determination. Finally, the final extracts were analyzed using [insert: GC-MS/MS or LC-MS/MS]. Quantification was carried out by comparing retention times and mass spectra with those of analytical standards. Calibration curves were prepared from matrix-matched standards at different concentrations

Recovery was determined by spiking blank maize samples (leaves) with known concentrations of pesticides and calculated as following:

Recovery (%)= C spiked / C measured ×100

Where: C is the concentration found after analysis, and C spiked is the known concentration added.

Physiological Measurements and biochemical assays :

Plant height and grain yield were measured as indicators of the effects of the tested pesticides on maize growth. Lipid peroxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay, following the method of Heath and Packer (2022). Antioxidant capacity was evaluated by the DPPH radical scavenging assay as described by Brand-Williams *et al.* (1995). Total phenolic content was quantified according to Campos *et al.* (2021) using a spectrophotometric assay.

Statistical analysis

Data were analyzed using a mixed-model ANOVA, with treatment considered as a fixed effect and block as a random effect, employing SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Mean comparisons were performed using Duncan's multiple range test at a significant differences ($p<0.05$).

RESULTS AND DISCUSSION

Residual analysis

The data in table (2) reveal that the residual concentrations declined progressively in leaves from 15 to 60 DAA across all chemical treatments. Chlorpyrifos residues decreased from 1.25 mg/kg at 15 DAA to 0.42 mg/kg at 30 DAA and 0.08 mg/kg at 60 DAA, with a half-life of 28.2 days, but still exceeded the MRL. Similarly, imidacloprid declined from 0.89 mg/kg (15 DAA) to 0.31 mg/kg (30 DAA) and 0.04 mg/kg (60 DAA), with a half-life of 22.7 days, also exceeding the MRL. Lambda-cyhalothrin showed a faster degradation pattern, decreasing from 0.63 mg/kg (15 DAA) to 0.18 mg/kg (30 DAA) and 0.02 mg/kg (60 DAA), with a shorter half-life of 17.9 days, and remained within the MRL.In contrast, no detectable residues were found in treatments with neem oil or *Bacillus thuringiensis* throughout the experimental period, indicating complete compliance with residue safety standards.

Table 2. Residual analysis in maize kernels after application (mg/kg ± SD)

Treatment	15 DAA	30 DAA	60 DAA	Half-life (d)	MRL Status
Chlorpyrifos	$1.25^{a} \pm 0.04$	$0.42^{a} \pm 0.02$	$0.08^{a} \pm 0.002$	28.2	Exceeded
Imidacloprid	$0.89^{b} \pm 0.03$	$0.31^{b} \pm 0.01$	$0.04^{b} \pm 0.001$	22.7	Exceeded
Lambda-cyhalothrin	$0.63^{c} \pm 0.02$	$0.18^{c} \pm 0.01$	$0.02^{c} \pm 0.001$	17.9	Compliant
Neem oil	ND	ND	ND	-	Compliant
B. thuringiensis	ND	ND	ND	-	Compliant

ND = Not Detected (<LSD 0.001 mg/kg); Different superscripts indicate significant differences (p<0.05)

The side effect on plant parameters

Data in table (3) show that the plant growth and productivity were significantly influenced by pesticide treatments. Chlorpyrifos caused the greatest reduction in both plant height (189.2 cm) and grain yield (7.42 t/ha), corresponding to a 24.5% yield reduction compared with the untreated control. Imidacloprid also suppressed growth and yield (195.6 cm; 7.85 t/ha), with a 20.1% reduction, though its effect was less severe than chlorpyrifos. Lambda-cyhalothrin showed a moderate impact, reducing yield by 14.3% relative to control. In contrast, neem oil and *Bacillus thuringiensis* maintained plant height (214.9 and 216.4 cm, respectively) and grain yield (9.48 and 9.64 t/ha, respectively) comparable to the control, with minimal yield reduction (3.6% and 1.9%). These findings indicate that synthetic insecticides, particularly chlorpyrifos and imidacloprid, impose phytotoxic effects that compromise crop performance, whereas biopesticides sustain crop growth and yield with negligible adverse impacts.

Table 3. the effect of tested materials on plant parameters.

Treatment	Plant Height (cm)	Grain Yield (t/ha)	Reduction (%)
Control	$218.3^{a} \pm 1.4$	$9.83^{a} \pm 0.12$	-
Chlorpyrifos	$189.2^{c} \pm 2.1$	$7.42^{c} \pm 0.15$	24.5
Imidacloprid	$195.6^{b} \pm 1.9$	$7.85^{b} \pm 0.14$	20.1
Lambda-cyhalothrin	$205.3^{b} \pm 1.7$	$8.42^{b} \pm 0.13$	14.3
Neem oil	$214.9^{ab} \pm 1.8$	$9.48^{b} \pm 0.14$	3.6
B. thuringiensis	$216.4^{a} \pm 1.6$	$9.64^{ab} \pm 0.13$	1.9

Different superscripts indicate significant differences (p<0.05)

Oxidative Stress Responses toward tested pesticides

Results in table (4) illustrate that the antioxidant activity and oxidative stress markers varied significantly among treatments. Chlorpyrifos caused the strongest oxidative stress, as reflected by the lowest DPPH scavenging activity (42.0%) and phenolic content (1.82 mg GAE/g), alongside the highest lipid peroxidation (MDA = 14.2 nmol/g). Imidacloprid also induced oxidative damage, with reduced antioxidant activity (44.3%) and elevated MDA (12.8 nmol/g), though less severe than chlorpyrifos. Lambda-cyhalothrin had moderate effects, maintaining intermediate antioxidant capacity (47.8%) and phenolics (2.08 mg GAE/g), with moderately high MDA (10.5 nmol/g).

In contrast, neem oil and *Bacillus thuringiensis* significantly enhanced antioxidant status, showing the highest DPPH scavenging (67.5% and 63.2%) and phenolic levels (3.03 and 2.89 mg GAE/g), while markedly lowering MDA accumulation (6.9 and 7.2 nmol/g). These results suggest that synthetic insecticides, particularly chlorpyrifos and imidacloprid, intensify oxidative stress, whereas biopesticides exert protective effects by boosting antioxidant defenses and minimizing lipid peroxidation.

Table 4. the effect of tested pesticides on antioxidant parameters.

Treatment	DPPH scavenging (%)	Phenolics (mg GAE/g)	MDA (nmol/g)
Control	$51.2^{b} \pm 1.3$	$2.15^{c} \pm 0.05$	$8.4^{c} \pm 0.3$
Chlorpyrifos	$42.0^{c} \pm 1.1$	$1.82^{d} \pm 0.04$	$14.2^{a} \pm 0.4$
Imidacloprid	$44.3^{c} \pm 1.2$	$1.91^{cd} \pm 0.04$	$12.8^{b} \pm 0.3$
Lambda-cyhalothrin	$47.8^{b} \pm 1.4$	$2.08^{c} \pm 0.05$	$10.5^{b} \pm 0.3$
Neem oil	$67.5^{a} \pm 1.6$	$3.03^{a} \pm 0.07$	$6.9^{d} \pm 0.2$
B. thuringiensis	$63.2^{a} \pm 1.5$	$2.89^{b} \pm 0.06$	$7.2^{d} \pm 0.2$

Different superscripts indicate significant differences (p<0.05)

The present findings on pesticide residues align with earlier studies emphasizing the risks of excessive pesticide use, which often leads to residue accumulation in food and long-term health concerns (Sanborn *et al*., 2004; DAF and FSAI, 2006). Variations in Maximum Residue Limits (MRLs) across countries (Codex, 2010) further complicate food safety, especially since no residue level can be considered entirely “safe” (Boobis *et al*., 2008). Several studies reported rapid dissipation of pesticides: cypermethrin residues on okra became undetectable after 17 days (Shinde *et al*., 2012), while carbaryl residues on brinjal dropped significantly within one day and reached safe levels after six days (Dhas and Srivastava, 2010; Kavadia and Shanker, 1976). Similarly, Deshmukh and Singh (1975) showed dissipation of carbaryl and malathion in okra. However, persistence varies across crops and compounds, as tomatoes treated with profenofos required eight days to reach safe levels, while pirimiphos-methyl was safer within one day (Abd Allah *et al*., 1993; Soliman, 1994). Radwan *et al*. (1995, 2001) also demonstrated variability, with residues exceeding MRLs in tomatoes but dissipating to safe levels in grapes after 21 days. These differences highlight the need to assess pesticide safety based on crop type and management system.

Moving to the Environmental stresses that may prompt various types of physiological response and oxidative damage in plants Parween *et al*., (2016). The pollutants in the environment are able to induce the intracellular over-production of reactive oxygen species (ROS), thus damaging plant cells. It is known that the reaction of such radicals with macromolecules particularly lipoprotein caused peroxidative damages more rapidly and is evident from membrane lipids destruction (Jan *et al*., 2012b). The present results are in partial agreement with previous findings. For instance, Parween (2012) observed that chlorpyrifos application in mungbean seedlings enhanced growth traits and yield components such as pod number, seed number, and dry seed weight, which contrasts with the present findings where chlorpyrifos caused growth suppression and yield reduction. Such differences could be attributed to differences in crop species, pesticide concentration, or environmental conditions.

Similarly, Chibu *et al*. (2002) and Boonlertnirum *et al*. (2005) reported that chitosan application stimulated plant growth and yield in rice and soybean, while Rehim *et al*. (2009) found comparable effects in maize and bean. These studies support the positive effects that observed in the present study with neem oil and *B. thuringiensis*, which maintained plant vigor and improved antioxidant status.

Conversely, the results of Amengor and Tetteh (2008) align more closely with recent findings, as they reported that increasing application rates of lindane and unden (propoxur) led to yield suppression in garden eggs and tomatoes, and only modest gains in okra. This pattern reflects the negative impact of synthetic pesticides at higher concentrations, consistent with the reduced growth and productivity observed under chlorpyrifos and imidacloprid in the current experiment. Parween *et al*. (2012) also found that foliar application of Chlorpyrifos at rate of 0–1.5 mM caused increase in Lipid peroxidation rate and proline content in day 20 whereas dehydroascorbate, oxidized and total glutathione were increased using the same rate in day 10. To conclude, the results suggest that while certain pesticides may provide temporary benefits under specific conditions, their prolonged or excessive use can negatively impact plant growth and yield. In contrast, biopesticides such as neem oil and *Bacillus thuringiensis* represent more sustainable alternatives, as they not only support plant performance but also strengthen antioxidant defenses, thereby reducing oxidative stress.

CONCLUSION

The findings indicate that synthetic pesticides such as chlorpyrifos, imidacloprid, and lambda-cyhalothrin leave varying levels of residues, with chlorpyrifos and imidacloprid exceeding the MRLs, while lambda-cyhalothrin remained compliant. These chemicals also negatively influenced plant growth, grain yield, and antioxidant status, with higher MDA levels and reduced DPPH scavenging activity. In contrast, neem oil and *Bacillus thuringiensis* showed no detectable residues, maintained plant height and yield close to the control, and significantly enhanced antioxidant capacity while reducing lipid peroxidation.

Overall, the results suggest that while certain pesticides may provide temporary benefits under specific conditions, their prolonged or excessive use can negatively impact plant growth and yield. In contrast, biopesticides such as neem oil and *Bacillus thuringiensis* represent more sustainable alternatives, as they not only support plant performance but also strengthen antioxidant defenses, thereby reducing oxidative stress.

Suggested applications: These findings emphasize the importance of promoting the use of biopesticides within crop protection programs. Regulatory agencies should reinforce residue monitoring and support safer pest management approaches. For farmers, gradually reducing dependence on synthetic pesticides and incorporating biopesticides into their practices can help protect crop yield and quality while also ensuring environmental and food safety.

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استخدام زيت النيم بكتريا الباسيلس ثرونوجنسيس كبدائل صديقة للبيئة للمبيدات المخلقة

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الملخص

أجريت هذه الدراسه بغرض معرفة درجة الاختلاف في كمية متبقي المبيد، معرفة مدي استجابة نبات الذرة المعامل بالمبيدات من حيث درجة النمو وكذلك التاثير التاكسدي لكل من مبيد الكلوروبيروفوس، الاميداكلوبيرد، اللمبادا سيهالوثرين ، زيت النيم و بكتريا الباسيلس ثرونوجنسيس علي النبات المعامل. أظهرت نتائج تحليل متبقيات المبيدات بقاء عالي لمتبقي مبيد الكلوربيروفوس و مبيد الاميدكلوبيرد لفتره تصل الي فترة نصف عمر ٢٨.٢ و ٢٢.٧ يوم علي التوالي، حيث ان كلاهما تعدي الحد اليومي المسموح لمتبقي المبيد. بالنسبة لمبيد اللمبادا سيهالوثرين بقي علي النبات المعامل حتي اليوم ١٧.٩ يوم و ذلك في حدود الأمان، بينما لم يتم رصد أي متبقيات لزيت النيم و بكتريا الباسيلس ثرونوجنسيس، و الذي يبيت مدي درجة امانهم علي البيئة. اما بالنسبة لدرجة نمو النبات ، فقد بينت النتائج ان هناك اختلافات بين المعاملات حيث أدت المعاملة بمبيد الكلوروبيرفوس الي انخفاض في طول النبات الي ١٨٩.٢ سم و الناتج أي ٧.٤٢ طن /هيكتار بما يعادل ٢٤.٥ انخفاض، يلي ذلك مبيد الاميدوكلوبيرد (١٩٥.٦ سم و ٧.٨٥ طن/هيكتار ٢٠.١ خفض) و مبيد اللمبادا سيهالوثرين (٢٠٥.٣ سم و ٨.٤٢ طن /هيكتار ب ١٤.٣٪ خفض) علي التوالي. علي النقيض، زيت النيم (٢١٤.٩سم ، ٩.٤٨طن/هيكتار و ٣.٦٪ خفض) و بكتريا الباسيلس ثرونوجنسيس (٢١٦.٣سم، ٩.٦٤ طن/هيكتار و ١.٩٥٪ خفض مقارنه بتجربة المقارنه. بالانتقال الي مضات الاكسده، و الذي اخذ نفس المنحني، حيث ادي مبيد الكلوروبيروفوس و الاميداكلوبيرد الي اثاره الاجهاد التاكسدي ، انخفاض في قدرة النبات علي إزالة الجزور الحره (٤٢.٠-٤٤٪)و المحتوي من حمض الفينوليك (١.٨٢-١.٩١ ملجم GAE /جم) بينما ادي الي زيادة في نشاط مالونديالدهيد (١٢.٨- ١٤.٢ نانومول/جم). كان لزيت النيم و بكتريا الباسيلس تأثير معاكس، حيث ادوا الي زيادة في نشاط المواد المضادة للاكسده (٦٧.٥٪ و ٦٣.٢٪) و الفينولات (٣.٠٣ و ٢.٨٩ ملجم GAE/جم)، بينما ادوا الي بقاء المواد المؤكسدة عند الحدود الدنيا. بشكل عام، تسببت المبيدات المخلقة الي حدوث نسب كبيرة من المتبقيات، الاجهاد على نبات الذرة، بينما المبيدات الحيوية اثبت مدي امانها علي النباتات المعاملة.