Misan Journal of Agricultural and Environmental Sciences

ARTICLE

Bioremediation of Emamectin Benzoate-Contaminated Soil by Indigenous Fungi: Degradation Kinetics and Restoration of Soil Carbon and Nitrogen Cycling

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ABSTRACT

The excessive application of Emamectin benzoate (EMB) can impair soil health and disrupt key biogeochemical processes in the living cells. This study evaluated the bioremediation potential of three indigenous fungal isolates including: *Alternaria infectoria*, *Lichtheimia corymbifera*, and *Aspergillus* fumigatus in EMB-contaminated soil under increasing pesticide stress up to 250 mg L^{-1}. The EMB degradation kinetics were determined by High-Performance Liquid Chromatography (HPLC), while soil respiration (CO_2 evolution) and urease activity were monitored as indicators of carbon and nitrogen cycling, respectively. The fungal inoculation significantly enhanced 100 mg L^{-1} EMB degradation, reducing the half-life from 4.70 days in the control to approximately 1.6 days. At the lethal concentration 250 mg L^{-1}, *A. infectoria* maintained the highest degradation rate k = 0.388 day^{-1} and the shortest half-life 1.79 days, compared with 5.84 days in the control. This isolate also produced the highest cumulative CO_2 emission 1680.9 mg kg^{-1} soil, indicating efficient EMB mineralization. In contrast, *A. fumigatus* showed the greatest capacity to restore nitrogen cycling by sustaining urease activity and overcoming the initial inhibition phase. Overall, *A. infectoria* exhibited the strongest bioremediation potential, highlighting its suitability for the remediation of EMB-contaminated agricultural soils.

Keywords: *First-order kinetics, Half-life, High-Performance Liquid Chromatography (HPLC), Soil respiration, Urease enzyme*

***Article extracted from the MSc thesis of the first researcher.**

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Citation: Abdulmunem, M. Q., Al-Jaberi, M. M., & Al-Farttoosy, A. H. (2026). Bioremediation of emamectin benzoate-contaminated soil by indigenous fungi: Degradation kinetics and restoration of soil carbon and nitrogen cycling. *Malabar Journal of Agriculture and Environment*. , *2*(2), 49-67. https://doi.org/10.64333/MJAE.26.2.6
Received: 08/05/2026
Accepted: 19/06/2026
Published: 30/06/2026
DOI: https://doi.org/10.64333/MJAE.26.2.6

INTRODUCTION

Microorganisms, particularly fungi, represent the fundamental engine for the sustaining soil health and fertility. They play a crucial role in the managing biogeochemical cycles, specifically the functional synergy between the carbon and nitrogen cycles. However, the intensive expansion of agricultural activities and the excessive reliance on pesticides such as the widely used compound Emamectin benzoate—have imposed severe toxic stress that threatens this ecological balance in agricultural lands. This environmental hazard is clearly manifested in the ability of these pesticides to paralyze extracellular enzymes, such as urease, which is considered the core backbone of nitrogen mineralization processes. The previous studies have demonstrated that chemical stress sharply reduces the enzyme-secreting microbial biomass and interferes with their active sites, leading to the deterioration of soil fertility and a decline in its productive efficiency (Baćmaga *et al*., 2015).

To evaluate the impact of this chemical stress, soil respiration and the release of carbon dioxide (CO_2) serve as the most prominent biological indicators for measuring microbial response and carbon cycling efficiency. Under the high pollution

levels, survival strategies vary; microbes are forced to elevate their metabolic quotient (qCO_2) and redirect their energy toward degrading the pollutant rather than building biomass (incurring a high metabolic cost). This creates complex respiratory patterns characterized by a biphasic behavior (Ashraf *et al*., 2022). This temporal kinetics reflects the transition of the microbial system from the inhibitory shock and lag phase to the recovery and complete mineralization phase of the pollutants, where the bioavailability of the pesticide gradually decreases, allowing the soil to restore its enzymatic activity (Akhtar and Mannan, 2020).

Despite the success of bioremediation techniques as sustainable environmental solutions, a precise understanding of the dynamics of microbial responses under lethal stress levels remains a significant research gap. Many remediation strategies fail in the field due to the oversight of crucial physiological phenomena, such as the "interaction masking effect" and the physiological trade-off between growth efficiency and cumulative stress tolerance (Malik *et al*., 2020). Hence an urgent action is needed to investigate the synergistic or individual efficiency of specialized fungal isolates capable of building mycelial networks and defensive shields. These structures create a clean microenvironment to protect degradative enzymes and form stabilized enzyme pools within soil colloids, ensuring the sustainability of the remediation over time (Chia *et al*., 2024).

Consequently, the current study has addressed the indigenous fungal isolates *Alternaria infectoria, Lichtheimia corymbifera,* and *Aspergillus fumigatus* to track the temporal kinetics of CO_2 emissions, measuring urease activity, and monitoring the EMB kinetics in the soil using High-Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS

Sample Collection

The soil samples were collected in November 2024 from the five different locations in Basra Governorate (southern and northern regions) for the purpose of isolating indigenous (local) fungi. These locations included: Al-Qurna, the Agricultural Research and Experiment Station (Al-Hartha District, University of Basra, Karma Ali), a palm grove in Abu Al-Khasib District, a vegetable farm in Al-Burjasiya area (Al-Zubair District), and experiments were conducted on soil collected from an orchard in Al-Faw District.

Soil Respiration Measurement

Soil samples from the study area (Al-Faw district) were utilized for this experiment. For the experimental setup, 250 g of soil were placed into glass containers and treated with Emamectin benzoate (EMB) at concentrations of 100, 125, and 250 mg L^{-1}. These levels corresponded to the Minimum Inhibitory Concentration (MIC), the Half-Maximal Inhibitory Concentration (IC_{50}), and the lethal concentration, respectively. The soil was subsequently inoculated with 10 mL of 3–7-day-old fungal isolate suspensions per treatment and moistened to field capacity. The experiment was conducted in triplicate for each treatment, alongside an uninoculated control, and incubated under controlled laboratory conditions. Soil respiratory activity was assessed by quantifying the emitted carbon dioxide (CO_2). A 1N NaOH solution was used to trap the liberated CO_2 after incubation periods of 3, 7, 14, 21, and 28 days. The total amount of evolved CO_2 was then calculated according to the method described by Ciardi and Nannipieri (1990) using the following equations:

$mEq\ CO^2 = (mEq\ NaOH - mEq\ HCl)$, and then $mg\ CO^2 = (mEq\ CO^2)(Equivalent\ weight)$. Where:

mEq: Milliequivalents.

Equivalent weight: The equivalent weight of carbon dioxide (which is 22).

Urease Enzyme Activity (Urease amidohydrolase, EC 3.5.1.5)

Soil urease activity was measured according to the method described by Tabatabai and Bremner (1972), utilizing urea as the specific substrate. Following the incubation period, the ammonium ion produced from the hydrolysis of urea was quantified via steam distillation using a Kjeldahl apparatus, in accordance with the protocol outlined by Bremner and Edwards (1965). and the enzyme activity was calculated using the following equation:

$$\frac{N(mg\ kg^{-1}) = (V_{HCl} - V_{Blank}) \times 0.005 \times 14}{1000} \times \frac{25}{10} \times \frac{100}{2.5} \times 10000$$

Where: $V_{HCl} - V_{Blank}$: Volume of HCl consumed during titration after subtracting the blank value (mL).

0.005: Normality of the HCl solution (N).

14: Atomic weight of nitrogen (g mol^{-1}), equivalent to 14 mg per milliequivalent (meq) of nitrogen.

1000: Conversion factor used to convert milligrams (mg) to grams (g).

25/10: Dilution factor, where 25 mL is the total digest volume and 10 mL is the aliquot used for distillation/titration.

100/2.5: Sample weight correction factor based on a 2.5 g soil sample.

10000: Conversion factor from percentage (%) to mg kg^{-1} (ppm), where 1% = 10,000 mg kg^{-1} (ppm).

Determination of Emamectin Benzoate Residues by High-Performance Liquid Chromatography (HPLC)

1-Sample Extraction

The extraction of EMB residues from the soil was performed following the protocol described by Kottiappan and Veilumuthu Anandh, (2012). Two grams of the soil sample were extracted with 200 mL of an acetone:water mixture (70:30, v/v) by shaking in a mechanical shaker for 2 hours. The contents were subsequently filtered. The pooled extract was partitioned with 100 mL of dichloromethane, and the lower organic layer was collected into a 500 mL round-bottom flask through anhydrous sodium sulphate. To the aqueous layer remaining in the separating funnel, 50 mL of saturated NaCl and 50 mL of dichloromethane were added. The mixture was shaken vigorously, and the lower organic layer was again collected through anhydrous sodium sulphate into the same 500 mL flask. This partitioning step was repeated with another 50 mL of dichloromethane. Finally, the combined dichloromethane extract was evaporated to dryness.

2-HPLC Analysis

The extracted residues were quantified utilizing an HPLC system, model SYKAM (Germany), equipped with a fluorescence detector. Chromatographic separation was achieved using a C18-ODS column (25 cm × 4.6 mm). The mobile phase consisted of acetonitrile and distilled water (95:5, v/v) at a constant flow rate of 1.0 mL min^{-1}. The fluorescence detector was operated with emission and excitation wavelengths of 365 nm and 460 nm, respectively.

Statistical Analysis

The experiments were structured as factorial experiments within a Completely Randomized Design (CRD), utilizing three replicates per treatment. Data analysis was executed via GenStat software (Release 12.1, VSN International Ltd., UK). Subsequently, the Least Significant Difference (LSD) test was employed to compare the treatment means at a significance level of 0.01.

RESULTS AND DISCUSSION

Soil collection

A five-soil location has been established to gather distinct locations in the Basra Governorate to isolate the fungi capable of using the EMB efficiently because of its inherent advantages for the various pesticide groups. (Figure 1) shows the sites where the soil sample was previously collected.

Figure (1) Represents the sites from which soils were taken for study

Soil Respiration and Carbon Mineralization Dynamics

Measurements of soil carbon dioxide (CO2) emissions, serving as a precise biogeochemical indicator of the biomonitoring and mineralization rates of organic pollutants, revealed a highly significant and differential response primarily dependent on the type of inoculated fungal isolate. The results demonstrated a significant superiority of the *Alternaria infectoria* (F2) isolate, which outperformed other treatments by recording the highest respiration rate (300.67 mg CO_2 kg^{-1} soil), representing a substantial increase of 115.6% compared to the uninoculated control (F0) (139.47 mg CO_2 kg^{-1} soil). This was followed by the *Aspergillus fumigatus* (F3) isolate with an increase of 95.9%, and the *Lichtheimia corymbifera* (F1) isolate with a significant increase of 69.9% relative to the control.

The comprehensive outperformance of these isolates confirms their high efficiency in utilizing Emamectin benzoate as a sole carbon source through the extensive secretion of extracellular enzymatic systems capable of cleaving its complex chemical rings. This behavior aligns with the findings of (Pujiati *et al*., 2024), who affirmed that the ultimate mineralization outcome is directly correlated with the specialized enzymatic capabilities of each microbial genus in the biotransformation of xenobiotics into safe end products.

In contrast to this pronounced biological effect, the statistical analysis revealed that the main effect of pesticide concentrations alone was statistically non-significant at the 0.01 significance level. This underscores the inadequacy of evaluating the concentration's impact in isolation from the biological agent.

The precise ecological and biological rationale for this lack of statistical significance becomes evident upon examining the two-way interaction between the fungal inoculation type and the EMB pesticide concentration (Figure

2A). This interaction reveals that the fungal isolates did not exhibit a uniform response to increasing toxic stress. Instead, they adopted entirely contrasting physiological strategies, leading to the neutralization of overall means and the obscuring of significant differences—a phenomenon ecologically recognized as the "interaction masking effect.

Specifically, the *A. infectoria* (F2) isolate demonstrated a highly positive proportional response to escalating stress. While it recorded a respiratory activity of 260.48 mg CO_2 kg^{-1} soil at the pesticide-free control concentration (P0), this activity increased progressively, peaking at the lethal concentration (P3) with 336.16 mg CO_2 kg^{-1} soil, representing a 29% increase. This significant elevation confirms the fungus's response to stress by expending additional energy to intensively synthesize degradation enzymes to counteract the severe pollution. Such a strategy characterizes highly stress-tolerant fungi, as elucidated by Mohapatra *et al*. (2022) in their comprehensive evaluation of fungal bioremediation efficiency. Their study highlighted the capability of soil fungi to serve as effective, nature-based bioremediation agents for degrading harmful agricultural pesticides and restoring soil health.

Conversely, the *A. fumigatus* (F3) isolate exhibited a fluctuation-resistant pathway, maintaining a clear, non-significant statistical convergence across all concentrations, ranging between 260.48 and 286.59 mg CO_2 kg^{-1} soil. This equilibrium reflects the stability of its dynamic enzymatic system and its resistance to inhibition with increasing toxic doses.

In a completely opposite response to isolate (F2), the *L. corymbifera* (F1) isolate displayed a typical response to toxic stress. It achieved its highest rate at the recommended concentration (P1) with an average of 247.87 mg CO_2 kg^{-1} soil, after which its activity gradually declined by 10.7%, reaching its lowest level at the lethal concentration (P3). This decline indicates the exhaustion and inhibition of its metabolic pathways at lethal concentrations. Such behavior is explained by van Bruggen *et al*. (2021) as a toxic shock induced by high concentrations of agrochemicals, which incompletely adapted microbial communities fail to cope with.

The ultimate outcome of these complex interactions clearly illustrates how the escalating behavior of isolate (F2), coinciding with the regressive behavior of isolate (F1) and the stability of isolate (F3), is the actual cause for the absence of significant differences when calculating the overall mean of pesticide concentrations. This fundamentally underscores the importance of studying biological interactions to understand the dynamics of bioremediation in contaminated soils.

The temporal kinetics of carbon dioxide (CO_2) emissions from soils treated with Emamectin benzoate reflect a complex dynamic pathway characterized by a biphasic behavior. By tracking the main effect of incubation periods, it is evident that the overall microbial activity recorded its highest respiratory peak at the onset of incubation on the third day, averaging 344.03 mg CO_2 kg^{-1} soil. This was followed by a sharp decline on the seventh day, reaching its lowest level (134.00 mg CO_2 kg^{-1} soil), before gradually recovering to record an excellent second peak on day 28 with an average of 286.18 mg CO_2 kg^{-1} soil.

Although this general trend is evident, a precise biogeochemical understanding of these kinetics remains incomplete without analyzing their underlying drivers through two-way interactions. The interaction between incubation time and fungal species (Figure 2B), as well as the interaction between time and pesticide concentrations (Figure 2C), reveal that this overarching curve is, in fact, the product of highly specialized physiological and toxicological responses.

When analyzing the early emission peak on the third day, the two-way interaction between fungal type and time demonstrates that this surge was not a random occurrence. Instead, it was driven by the intense respiratory activity of the active isolates, specifically *A. fumigatus* (F3) and *A. infectoria* (F2), which recorded 450.67 and 442.93 mg CO_2

kg⁻¹ soil on the third day, respectively.

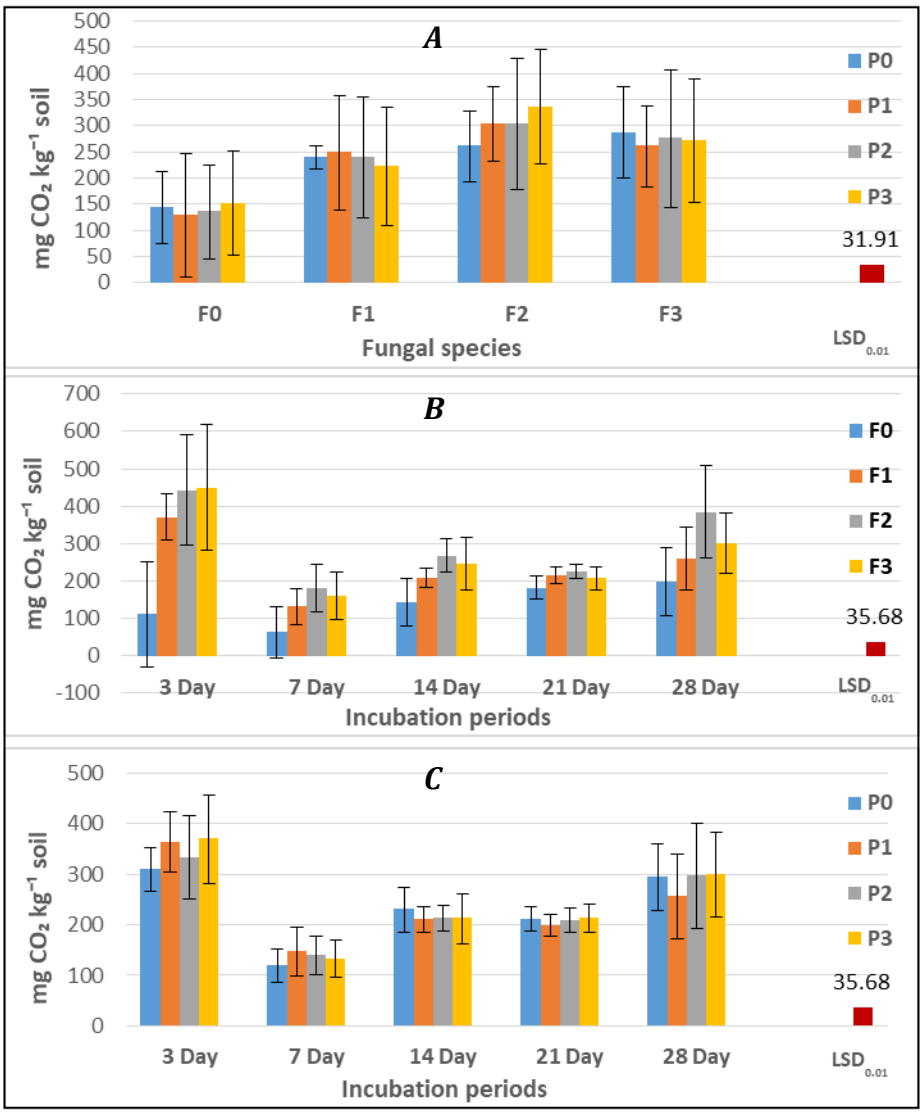

Figure (2) Effect of two-way interactions on the amount of CO_2 released from the soil. (A), Interaction between fungal species and pesticide concentrations. (B), Interaction between fungal species and incubation time. (C), Interaction between pesticide concentrations and incubation time.
Where P0= no EMB, P1= EMB at the first concentration = 100 mg L^{-1}, P2= EMB at the second concentration = 125 mg L^{-1}, and P3= EMB at the third concentration = 250 100 mg L^{-1}.

The role of toxic stress in this surge becomes apparent when examining the interaction between concentrations and time on the same day. Microbial activity increased at the lethal concentration (P3) to record 369.63 mg CO_2 kg^{-1} soil, an increase of 19.4% compared to the control (P0). This temporal coincidence biologically substantiates the occurrence of enhanced enzymatic activity as an immediate response to initial exposure to high pesticide concentrations (Riah *et al*., 2014). At this stage, fungi incur a high metabolic cost, redirecting their energy toward breaking down and cleaving the xenobiotic compound before it penetrates their cells, rather than building biomass. This is ecologically known as an elevated metabolic quotient (qCO_2) under conditions of toxic stress (Ashraf *et al*., 2022).The efficiency of bioremediation is manifested in the phase of complete physiological adaptation, which became clearly evident on day

28 (Figure 2B).

The two-way interactions between fungal type and time revealed recovery capabilities that successfully surpassed the toxic shock. Isolate (F2) surged again to record 385.00 mg CO_2 kg^{-1} soil, achieving a substantial recovery rate of 112.5% from its lowest point. This biological recovery was accompanied by a parallel recovery at maximum stress levels, as activity at the lethal concentration (P3) rose to 299.20 mg CO_2 kg^{-1} soil, registering a recovery rate of 127.3% compared to the seventh day. This delayed alignment between the peak of isolate (F2) and the peak of concentration (P3) strongly corroborates the transition of the soil biological system from the toxicity-combating phase to the full mineralization phase. During this phase, fungi completed the synthesis of their adaptive enzymes and utilized the pesticide as a sustainable energy source (Vaksmaa *et al*., 2023). This embodies the exceptional functional resilience of fungal communities in harsh environments, which is consistent with the functional resilience framework proposed by Schaeffer *et al*., (2016).

Linking the periodic measurements of respiration rates across the incubation periods (Table 1), representing the three-way interaction) with the final cumulative carbon dioxide outcome (Table 2) provides the most comprehensive picture for evaluating the bioremediation potential of the fungal isolates against the EMB pesticide. While periodic measurements reveal the mechanisms of physiological adaptation and resistance to toxic shocks, the total CO_2 accumulation (Table 2) translates this resistance into a final mineralization outcome that accurately reflects the magnitude of the degraded pollutants.

The *A. infectoria* (F2) isolate demonstrated exceptional superiority, combining a rapid response with cumulative tolerance. As detailed in (Table 1), when confronting the lethal concentration (P3), this isolate exhibited an early respiratory surge on the third day (451.73 mg CO_2 kg^{-1} soil), indicative of enhanced enzymatic activity. Despite experiencing a toxic shock and a subsequent decline on the seventh day, it displayed remarkable resilience, achieving a second recovery peak of 325.60 mg CO_2 kg^{-1} soil on day 28. This active kinetic behavior translated into a substantial cumulative outcome by the end of the incubation period (Table 2), where the isolate recorded the highest total CO_2 accumulation at the lethal concentration (P3), achieving 1680.9 mg CO_2 kg^{-1} soil. Where F0= no fungus, F1= *Alternaria infectoria*, F2= *Lichtheimia corymbifera*, F3= *Aspergillus fumigatus*, and P0= no EMB, P1= EMB at the first concentration = 100 mg L^{-1}, P2= EMB at the second concentration = 125 mg L^{-1}, and P3= EMB at the third concentration = 250 100 mg L^{-1}. Comparing this figure with the abiotic emission of the uninoculated control (F0) at the same concentration (756.8 mg CO_2 kg^{-1} soil), it is evident that the fungus achieved a remarkably high cumulative increase of 122.1%. This pathway of the three-way interaction demonstrates the A. *infectoria* (F2) isolate demonstrated exceptional superiority, combining a rapid response with cumulative tolerance. As detailed in (Table 1), when confronting the lethal concentration (P3), this isolate exhibited an early respiratory surge on the third day (451.73 mg CO_2 kg^{-1} soil), indicative of enhanced enzymatic activity. Despite experiencing a toxic shock and a subsequent decline on the seventh day, it displayed remarkable resilience, achieving a second recovery peak of 325.60 mg CO_2 kg^{-1} soil on day 28. This active kinetic behavior translated into a substantial cumulative outcome by the end of the incubation period (Table 2), where the isolate recorded the highest total CO_2 accumulation at the lethal concentration (P3), achieving 1680.9 mg CO_2 kg^{-1} soil. Comparing this figure with the abiotic emission of the uninoculated control (F0) at the same concentration (756.8 mg CO_2 kg^{-1} soil), it is evident that the fungus achieved a remarkably high cumulative increase of 122.1%. This pathway of the three-way interaction demonstrates that the fungus did not merely resist toxicity but completely reprogrammed its metabolic pathways to exploit the pesticide as a sustainable carbon source. This aligns with the biodegradation mechanisms supporting the ability of the *Alternaria* genus to degrade pesticides and utilize

them for growth (Kadhim, 2014).

Table (1) Effect of the three-way interaction among fungal species, pesticide concentrations, and time on CO_2 released from the soil (mg CO_2 kg^{-1} soil).

Fungal species F	Pesticide concentrations P	Time (Days)				
		3	7	14	21	28
F0	P0	105.60	46.93	170.13	187.73	202.40
	P1	322.67	66.00	198.00	214.13	397.47
	P2	401.87	202.40	275.73	202.40	220.00
	P3	407.73	158.40	275.73	237.60	353.47
F1	P0	108.53	117.07	126.13	161.33	132.00
	P1	448.80	162.80	217.07	208.27	202.40
	P2	431.20	146.67	278.67	237.60	422.40
	P3	466.40	162.80	220.00	189.20	264.00
F2	P0	111.47	44.00	158.40	193.60	167.20
	P1	365.20	170.13	234.67	205.33	220.00
	P2	404.80	176.00	234.67	222.93	475.20
	P3	451.73	164.27	222.93	211.20	325.60
F3	P0	120.27	44.00	117.33	184.80	290.40
	P1	347.60	129.07	180.40	228.80	220.00
	P2	533.87	199.47	281.60	243.47	422.40
	P3	476.80	154.00	268.40	190.67	264.00
$LSD_{0.01}$ = 71.36						

Table (2) Cumulative CO_2 released from the soil (mg CO_2 kg^{-1} soil) as affected by the three-way interaction among fungal species, pesticide concentrations, and time impact of Azolla extract, varieties, and interaction among on flag leaf area (cm2).

Fungal species F	Pesticide concentrations P	Time (Days)				
		3	7	14	21	28
F0	P0	105.6	152.5	322.6	510.3	712.7
	P1	108.5	225.6	351.7	513.0	645.0
	P2	111.5	155.5	313.9	507.5	674.7
	P3	120.3	164.3	281.6	466.4	756.8
F1	P0	322.7	388.7	586.7	800.8	1198.3
	P1	448.8	611.6	828.7	1037.0	1239.4
	P2	365.2	535.3	770.0	975.3	1195.3
	P3	347.6	476.7	657.1	885.9	1105.9
F2	P0	401.9	604.3	880.0	1082.4	1302.4
	P1	431.2	577.9	856.6	1094.2	1516.6
	P2	404.8	580.8	815.5	1038.4	1513.6
	P3	533.9	733.4	1015.0	1258.5	1680.9
F3	P0	407.7	566.1	841.8	1079.4	1432.9
	P1	466.4	629.2	849.2	1038.4	1302.4
	P2	451.7	616.0	838.9	1050.1	1375.7
	P3	476.8	630.8	899.2	1089.9	1353.9
$LSD_{0.01}$ = 71.36						

Where F0= no fungus, F1= *Alternaria infectoria*, F2= *Lichtheimia corymbifera*, F3= *Aspergillus fumigatus*, and P0= no EMB, P1= EMB at the first concentration = 100 mg L^{-1}, P2= EMB at the second concentration = 125 mg L^{-1}, and P3= EMB at the third concentration = 250 mg L^{-1}.

Conversely, the *Aspergillus fumigatus* (F3) isolate followed a kinetic pathway characterized by physiological stability. The isolate maintained a balanced recovery pattern during the later incubation periods without severe fluctuations (Table 1). This stability was directly reflected in the cumulative mineralization data (Table 2), where the isolate achieved statistically convergent totals across all concentrations by the end of the experiment (1432.9, 1302.4, 1375.7, and 1353.9 mg CO_2 kg^{-1} soil for the P0, P1, P2, and P3 concentrations, respectively).

This cumulative convergence and the absence of a decline in the final outcome with increasing pesticide doses provide conclusive evidence that this isolate possesses a dynamic enzymatic system resistant to cumulative inhibition, rendering it an ideal "stable degrader." This behavior is highly consistent with the findings of (Matúš *et al*., 2023), who confirmed that this genus possesses highly tolerant extracellular enzymatic systems capable of maintaining stable mineralization levels even in severely contaminated environments.

In contrast, linking the periodic measurements of emitted CO_2 reveals the physiological tolerance limits of the *L. corymbifera* (F1) isolate. Despite its high dynamic activity during the initial days at the recommended concentration (P1), tracking its cumulative trajectory (Table 2) revealed a diminished capacity to sustain this activity under high toxicity levels. The isolate achieved its optimal cumulative performance at (P1), recording 1239.4 mg CO_2 kg^{-1} soil; however, this total declined to 1105.9 mg CO_2 kg^{-1} soil at the lethal concentration (P3).

This cumulative reduction of 10.8% serves as physiological evidence that the isolate entered a state of "chronic metabolic stress." The prolonged exposure to lethal doses and their intermediate degradation byproducts resulted in the depletion of the fungal energy reserves and the exhaustion of its mineralization pathways. This interpretation is corroborated by Meena *et al*. (2020), who found that elevated concentrations of agrochemicals induce environmental stress, leading to a decline in the cumulative capacity of incompletely adapted microbes.

The ultimate conclusion drawn from these complex interactions asserts that the success of bioremediation is not solely gauged by the activity observed in the early stages, but rather by the capability to sustain mineralization until the pollutant is entirely degraded, as elucidated by Pang *et al*. (2020). Consequently, the A. *infectoria* (F2) isolate stands out as the most potent biological candidate among the investigated species for remediating heavily contaminated agricultural hotspots, owing to its superior cumulative capacity.

Urease Activity (Urease amidohydrolase, EC 3.5.1.5)

The results regarding the main effect of EMB pesticide concentrations demonstrated a clear variation in soil urease activity, revealing a highly significant inverse and inhibitory response at the 0.01 significance level with increasing pesticide concentrations. The pesticide-free control treatment (P0) recorded the highest mean enzyme activity, reaching 120.11 mg NH_4^+ kg^{-1} soil. Conversely, the addition of the pesticide at the field-recommended concentration (P1) induced considerable stress, reducing the activity to 88.38 mg NH_4^+ kg^{-1} soil, which represents a decrease of approximately 26.4% compared to the control.

This sharp downward trend continued, with the enzyme activity plummeting to its lowest level at the lethal concentration (P3), recording 45.85 mg NH_4^+ kg^{-1} soil. This substantial decline indicates a loss of approximately 61.8% of the original soil enzymatic activity, profoundly reflecting the severity of the chemical stress exerted on the biological system.

This high inhibition in enzymatic activity can be attributed to the direct toxic effect of the EMB pesticide on the enzyme-secreting microbial biomass. This aligns with the findings of Riah *et al*. (2014), who explained that the chemical pressure of pesticides exerts negative inhibitory effects on microbial activity, consequently reducing the

accumulation of extracellular enzymes. Furthermore, as elucidated by Stoytcheva, (2011) in a comprehensive assessment of pesticide impacts, chemical stress leads to the interference of the pollutant's active ingredient or its degradation byproducts with the enzyme's active sites. This interference severely impedes the enzyme's binding to its specific substrate, thereby drastically restricting nitrogen mineralization processes.

Although this inhibition inherently reflects a biological stress, from an applied agricultural perspective, it may carry an indirect positive aspect. The reduction in soil urease activity resulting from pesticide application decelerates the rapid hydrolysis of urea fertilizers. This phenomenon is generally considered beneficial, as it mitigates nitrogen loss through volatilization and prolongs its availability in the soil for plant uptake (Antonious, 2003).

Bio-inoculation emerged as a highly effective ecological solution for stimulating soil metabolic processes. The results concerning the main effect of fungal isolates demonstrated a clear, highly significant superiority at the 0.01 significance level of all bio-inoculation treatments in enhancing urease activity compared to the uninoculated control (F0). The fungal isolate *A. fumigatus* (F3) exhibited the highest performance, recording a maximum mean enzyme activity of 97.52 mg NH_4^+-N kg^{-1} soil $2h^{-1}$, thereby achieving a substantial increase of approximately 55.5% relative to the control, which recorded only 62.72 mg NH_4^+-N kg^{-1} soil $2h^{-1}$. The *Alternaria infectoria* (F2) isolate ranked second with an activity of 86.38 mg NH_4^+-N kg^{-1} soil $2h^{-1}$ (a 37.7% increase), whereas the *L. corymbifera* (F1) isolate exhibited the lowest activity among the evaluated fungi, averaging 76.50 mg NH_4^+-N kg^{-1} soil $2h^{-1}$ (a 22% increase).

This elevation in urease activity following fungal inoculation can be attributed to the capacity of filamentous fungi to develop an extensive mycelial network within soil pore spaces. This network expands the surface area and creates favorable physicochemical conditions that amplify fungal activity in degrading organic compounds (i.e., the pesticide), thereby establishing a clean microenvironment devoid of chemical stress. Consequently, this promotes the fungal capacity to synthesize the enzyme and excrete it into the soil matrix. This mechanistic interpretation is consistent with the findings of Harms *et al*. (2011) regarding fungal-mediated bioremediation, which highlighted that the penetrative capacity and spatial proliferation of fungal mycelia in the soil significantly enhance the degradation efficiency of organic pollutants.

The variation in efficiency among the isolates is fundamentally attributed to their inherent taxonomic and physiological differences. The overwhelming superiority of the *A. fumigatus* (F3) isolate stems from its classification as an advanced ascomycete (Ascomycota). These fungi possess highly sophisticated enzymatic systems and are considered among the most critical primary decomposers of soil organic matter. Its high genetic capacity for the prolific secretion of the urease enzyme renders it a formidable functional competitor in nitrogen mineralization processes, a trait corroborated by the molecular analysis study of Xiong *et al*. (2023).

Following in the efficiency is the *A. infectoria* (F2) isolate, which demonstrated excellent competitive ability. Although it is a dematiaceous fungus (darkly pigmented), its exceptional adaptation to the local soil environment enabled it to maintain stable and effective enzyme production. making them highly competitive and stable in the exploitation of soil resources.

In contrast, the relatively lower response of the *L. corymbifera* (F1) isolate is ascribed to its classification as a zygomycete (Zygomycota). Ecologically, this group is categorized as "sugar fungi" or fast-growing primary colonizers that exhibit a strong preference for simple carbon sources. Despite their rapid vegetative growth, their capacity for the sustainable hydrolysis of complex nitrogenous compounds (and the subsequent secretion of urease) is generally lower when compared to advanced and specialized fungal genera such as *Aspergillus*. This limitation aligns with the findings of Leifheit *et al*. (2024), who confirmed that zygomycetes lack the complex enzymatic capabilities inherent to

ascomycetes. The latter possess advanced enzymatic mechanisms and functionally excel in the sustainable secretion of extracellular enzymes, thereby explaining the placement of *L. corymbifera* in the lowest rank among the inoculated isolates.

Given that physiological performance and the accumulation of secreted enzymes constitute a kinetic process, it was imperative to monitor this activity over time. The results concerning the main effect of incubation periods demonstrated a positive and progressive temporal evolution in enzyme activity. The 30-day incubation period exhibited a clear, highly significant superiority at the 0.01 significance level, recording a mean activity of 95.93 mg NH_4^+-N kg^{-1} soil $2h^{-1}$ compared to the 15-day period, which recorded 65.63 mg NH_4^+-N kg^{-1} soil $2h^{-1}$, representing a substantial increase of approximately 31.6%.

This significant elevation can be attributed to the fact that an extended incubation period affords sufficient time for the isolates to acclimatize and develop an extensive mycelial network. Furthermore, it facilitates the binding of the secreted enzymes to soil colloids, shielding them from rapid proteolytic degradation by other soil enzymes and thereby creating a stabilized enzyme pool. This mechanistic interpretation is highly consistent with the conceptual framework proposed by Burns *et al*. (2013). Additionally, this upward trajectory reflects a state of microbial recovery and a gradual decline in the bioavailability and toxicity of the pesticide following the initial inhibitory toxic shock (Hussain *et al*., 2009).

Having established the general behavior of each factor individually, a precise understanding of the remediation mechanism necessitates evaluating the direct interaction between the biological agent and chemical stress. Consequently, the two-way interaction between fungal isolates and pesticide concentrations revealed significant variations in enzyme activity, reflecting distinct resistance strategies (Figure 3A). In the absence of pesticide-induced chemical stress (P0), the *A. fumigatus* (F3) isolate exhibited superior performance (144.88), followed by *L. corymbifera* (F1) (116.08). However, upon exposure to advanced concentration levels (P2), a sharp decline in the enzymatic activity of both (F3) and (F1) isolates was observed, dropping to 71.77 and 74.08, respectively. In contrast, the *A. infectoria* (F2) isolate maintained the highest enzymatic activity at this concentration, recording 83.40, with its activity exhibiting a mere 11.6% reduction when transitioning from the control to the (P1) concentration.

This interaction provides concrete empirical evidence of the physiological trade-off between growth and defense. Fast-growing and enzymatically prolific isolates channel the majority of their energy toward spatial proliferation, thereby increasing their susceptibility to toxicity. This phenomenon is consistent with the ecological framework proposed by Malik *et al*. (2020) regarding soil microbial strategies, which highlighted a fundamental trade-off between growth efficiency and stress tolerance. Microorganisms that prioritize rapid vegetative growth often lack adequate protective mechanisms under severe chemical stress conditions. Conversely, the remarkable tolerance exhibited by isolate (F2) elucidates its previously noted lower overall enzymatic yield; this isolate strategically invests its energy into synthesizing defensive shields, particularly melanin. Melanin possesses a superior capacity to adsorb toxins and safeguard the internal cellular and enzymatic machinery against chemical damage (Cordero and Casadevall, 2017).

Fungal resistance assumes time-varying pathways that reflect the dynamics of recovery. Through the results of the interaction between concentration levels and incubation periods (Figure 3B), distinct responses are evident, illustrating the kinetics of pesticide degradation and microbial recovery. During the initial period (15 days), the addition of the pesticide induced a pronounced inhibitory shock; urease activity dropped sharply at the moderate inhibitory concentration (P2), recording only 43.04 compared to the control (111.52).

However, the most critical physiological turning point emerged after 30 days of incubation. The (P2) treatment witnessed a massive recovery leap, with its enzymatic activity reaching 94.49—meaning the enzyme activity more than doubled, increasing by over 119% compared to the initial period. This substantial recovery clearly indicates that the bioavailability and toxicity of moderate stress levels decrease over time as a result of the adsorption and biodegradation processes of the pesticide in the soil matrix. The alleviation of this toxic stress permitted the microbial community to overcome the shock phase and restore its vital activity in secreting the urease enzyme for nitrogen mineralization.

This dynamic aligns precisely with the scientific review by Akhtar and Mannan, (2020) on mycoremediation. They elucidated that fungi possess a high adaptive capacity to break the initial lag phase caused by pollutant toxicity. Once the fungus begins to dismantle the chemical xenobiotic and diminish its toxic stress over time, it profusely resumes the secretion of its extracellular enzymes to compensate for the prior deficit and restore the biochemical balance within the soil microenvironment.

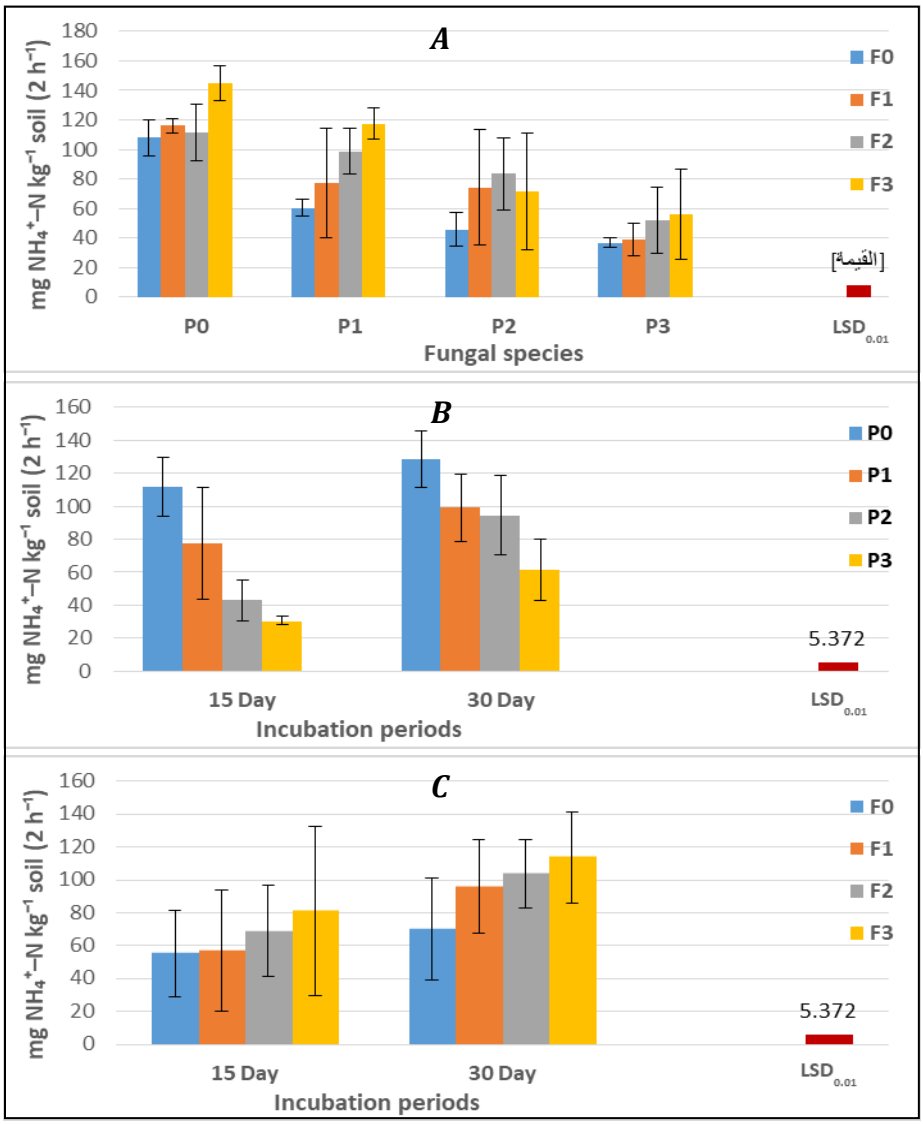

Figure (3) Effect of two-way interactions on urease activity in soil. (A), Interaction between fungal inoculation and pesticide concentrations. (B), Interaction between pesticide concentrations and incubation time. (C), Interaction between fungal inoculation and incubation time. Where F0= no fungus, F1= *Alternaria infectoria*, F2= *Lichtheimia corymbifera*, F3= *Aspergillus fumigatus*, and P0= no EMB, P1= EMB at the first concentration = 100 mg L^{-1}, P2= EMB at the second concentration = 125 mg L^{-1}, and P3= EMB at

the third concentration = 250 100 mg L^{-1}.

The results of the two-way interaction between incubation periods and fungal isolates (Figure 3C) revealed an ascending trajectory that reflects the kinetics of recovery and enzyme accumulation across the different fungal species. The *Aspergillus fumigatus* (F3) isolate maintained its forefront position as the most robustly active isolate, with its activity increasing from 81.23 (at 15 days) to reach the peak of nitrogen mineralization at 113.81 (at 30 days). Meanwhile, the *L. corymbifera* (F1) isolate exhibited a remarkable recovery surge, as its enzymatic activity escalated from 57.04 (a value not significantly different from the initial uninoculated control) to 95.95, thereby recording the highest temporal increase rate of approximately 68.2%.

This positive temporal evolution is attributed to the inherent nature of urease as an extracellular enzyme. Over time, and upon overcoming the initial stress shock, the fungal isolates begin to expand their mycelial networks and secrete increasing quantities of enzymes. These secreted enzymes do not degrade rapidly; rather, they bind to soil colloids and humus components. This interaction leads to the formation of what is known as a "stabilized enzyme pool," which accumulates over time and elevates the overall enzymatic activity of the soil. This is corroborated by the comprehensive review by Chia *et al*. (2024) on the role of microbial enzymes in the remediation of pesticide-contaminated soils. They elucidated that microorganisms, such as fungi, possess high efficiency in secreting extracellular degradative enzymes that accumulate and stabilize within the soil environment, thereby ensuring the sustainability of pollutant dismantling processes and the mitigation of pesticide toxicity over time.

The ecological and physiological picture is completed by analyzing the overall combined response. The results of the three-way interaction (Table 3) revealed a dynamic interplay between the intensity of stress and temporal adaptive capacity. The treatment comprising isolate (F3) at 30 days without pesticide recorded the peak enzyme activity (155.5). However, the applied significance is most pronounced under elevated concentrations. The activity of isolate (F1) at the moderate concentration (P2) plummeted during the first 15 days (38.5), yet it exhibited a distinctive recovery surge after 30 days, rising to 109.67. This provides concrete evidence for a "delayed enzyme induction" mechanism, where energy is initially directed toward detoxification before reverting to urease secretion (Rodríguez-Rodríguez *et al*., 2017). The results culminate at the lethal concentration (P3). Following a near-total paralysis of all isolates during the first 15 days of incubation, the *A. fumigatus* (F3) isolate demonstrated its absolute competence by successfully overcoming the toxicity barrier and boosting its activity to 84 by the thirtieth day. This underscores its remarkable enzymatic resilience and its capability to exploit the partial degradation products of the pesticide even in the harshest chemical environments over time.

Table (3) Effect of the three-way interaction among fungal inoculation, pesticide concentrations, and time on soil urease activity (mg NH_4^+–N kg^{-1} soil 2 h^{-1}).

Fungal isolate	**F0**		**F1**		**F2**		**F3**	
Time (Days) / Concentration	**15**	**30**	**15**	**30**	**15**	**30**	**15**	**30**
P0	96.83	119	117.83	114.33	97.15	126	134.27	155.5
P1	55	65.83	43.2	110.8	85.2	112	126.6	108.4
P2	35.65	55.97	38.5	109.67	61.8	105	36.2	107.33
P3	33.8	39.67	28.63	49	31.53	72.33	27.83	84
$LSD_{0.01}$ = 10.743								

Where F0= no fungus, F1= *Alternaria infectoria*, F2= *Lichtheimia corymbifera*, F3= *Aspergillus fumigatus*, and P0= no EMB, P1= EMB at the first concentration = 100 mg L^{-1}, P2= EMB at the second concentration = 125 mg L^{-1}, and P3= EMB at the third concentration =

250 100 mg L^{-1}.

Biodegradation Kinetics of Emamectin Benzoate (EMB) Residues in Soil

(Figure 4) illustrates the kinetic behavior of the degradation of Emamectin benzoate (EMB) residues in the soil at the recommended concentration (100 mg L^{-1}). The curve for the uninoculated control treatment (EMB only) displays a gradual and slow linear decline. This is corroborated by the final results in (Table 4), where the degradation rate constant (k) reached approximately 0.148 day^{-1}, taking 4.70 days for the half of the initial EMB concentration to degrade ($t_{1/2}$). This slow deterioration reflects natural abiotic degradation and limited indigenous microbial activity. It aligns with the chemical nature of the EMB, which is characterized by its high soil adsorption, thereby restricting its mobility and rendering its natural elimination a slow process (Chukwudebe *et al*., 1997).Furthermore, (Figure 4) demonstrates a very sharp and rapid decline in the curves associated with the biological treatments during the first seven days, confirming the high degradation efficiency of the fungal isolates. (Table 4) revealed a remarkable superiority of the *Lichtheimia corymbifera* and *Alternaria infectoria* fungi, which recorded the highest degradation rate constants (0.430 and 0.427 day^{-1}, respectively), while the half-life of the EMB was substantially reduced to approximately 1.6 days. This functional superiority can be attributed to the capacity of these fungi to secrete a comprehensive system of extracellular enzymes capable of accessing the pesticide molecules and cleaving their complex ester bonds (Berestetskiy and Hu, 2021). The strong consistency of these results with the high determination coefficient (R^2) values presented in (Table 4) confirms that the biodegradation process strictly adheres to the first-order kinetics model (Rajesh *et al*., 2024).

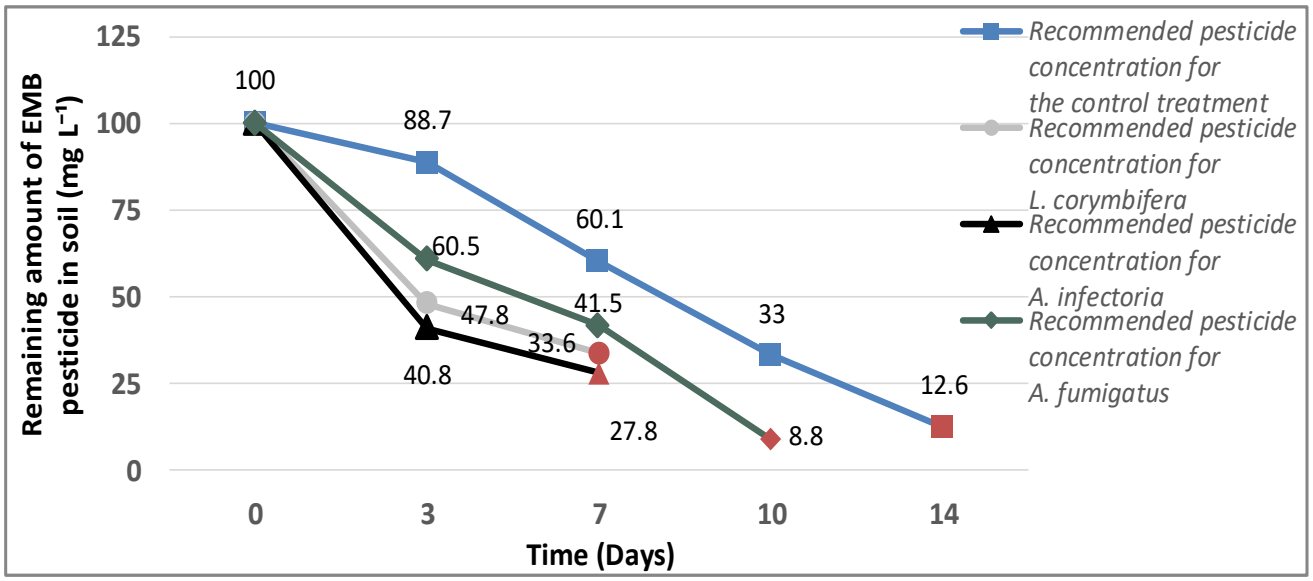

Figure (4) Kinetic behavior of the degradation of Emamectin Benzoate (EMB) residues in soil at the recommended concentration (100 mg L^{-1}).

Table (4) First-order degradation kinetic constants and half-life ($t_{1/2}$) of the pesticide, determined by HPLC at different concentrations under the influence of the studied fungal isolates.

Pesticide concentration (mg L^{-1})	Fungal species	Degradation rate constant (k) (day^{-1})	Half-life ($t_{1/2}$) (days)	Coefficient of determination (R^2)
100	*L. corymbifera*	0.430	1.61	0.824
	A. infectoria	0.427	1.62	0.844
	A. fumigatus	0.361	1.92	0.862
	Control (just pesticide)	0.148	4.70	0.923
125	*L. corymbifera*	0.442	1.57	0.854
	A. infectoria	0.437	1.59	0.868
	A. fumigatus	0.353	1.97	0.790
	Control (just pesticide)	0.129	5.38	0.971
250	*L. corymbifera*	0.220	3.15	0.925
	A. infectoria	**0.388**	**1.79**	**0.777**
	A. fumigatus	0.180	3.85	0.942
	Control (just pesticide)	0.119	5.84	0.981

Upon analyzing the kinetic behavior of the EMB under a higher level of chemical stress in the soil at a concentration of 125 mg L^{-1}, (Figure 5A) illustrates a further reduction in the natural degradation rate of the control treatment. According to the final results in (Table 4), the degradation rate constant dropped to 0.129 day^{-1}, and the half-life extended to 5.38 days. This occurs because an increased concentration of pesticide residues enhances its environmental persistence and hinders abiotic degradation processes (Fenner *et al*., 2013). In contrast, the fungal isolates demonstrated remarkable physiological resilience, as visually confirmed in (Figure 5A), with the biodegradation curves maintaining their steep decline. The statistical values presented in (Table 4) prove that the fungi *L. corymbifera* and *A. infectoria* not only maintained their efficiency but also exhibited high kinetic

stability, achieving degradation rates of 0.442 and 0.437 day^{-1}, respectively. This physiological stability is scientifically explained by the phenomenon of "enzyme induction." At this specific concentration, the toxic threshold capable of inhibiting the fungal mycelium had not yet been reached. Instead, the moderate stress acted as an inducer, upregulating the gene expression of toxin-digesting enzymes capable of cleaving the ester bonds within the pesticide's molecular structure (Harms *et al*., 2011). This behavior, documented both visually and statistically, corroborates the phenotypic plasticity of these isolates. Their inherent tolerance mechanisms strongly position them as promising biological candidates for the remediation of severely contaminated environments (Pinedo-Rivilla *et al*., 2009).

Upon exposing the fungal isolates to the high and lethal concentration of 250 mg L^{-1}, (Figure 5B) displayed a radical shift, revealing profound physiological differences in chemical stress tolerance. The kinetic constants documented in (Table 4) indicate the occurrence of a "toxic shock" that inhibited the efficiency of enzyme induction. (Figure 5B) illustrates a noticeable regression in the curve of the *L. corymbifera* fungus, where its degradation rate in (Table 4) dropped to 0.220 day^{-1} and the pesticide's half-life doubled to 3.15 days. Similarly, the *A. fumigatus* fungus declined, recording 0.180 day^{-1}.This evident inhibition observed in these fungi is attributed to the pesticide concentration exceeding their physiological tolerance threshold. Recent studies have demonstrated that high doses of Emamectin benzoate induce a toxic stress that leads to the inhibition of spore germination and a decline in the mycelial growth of vital soil fungi (Zhang *et al*., 2022).

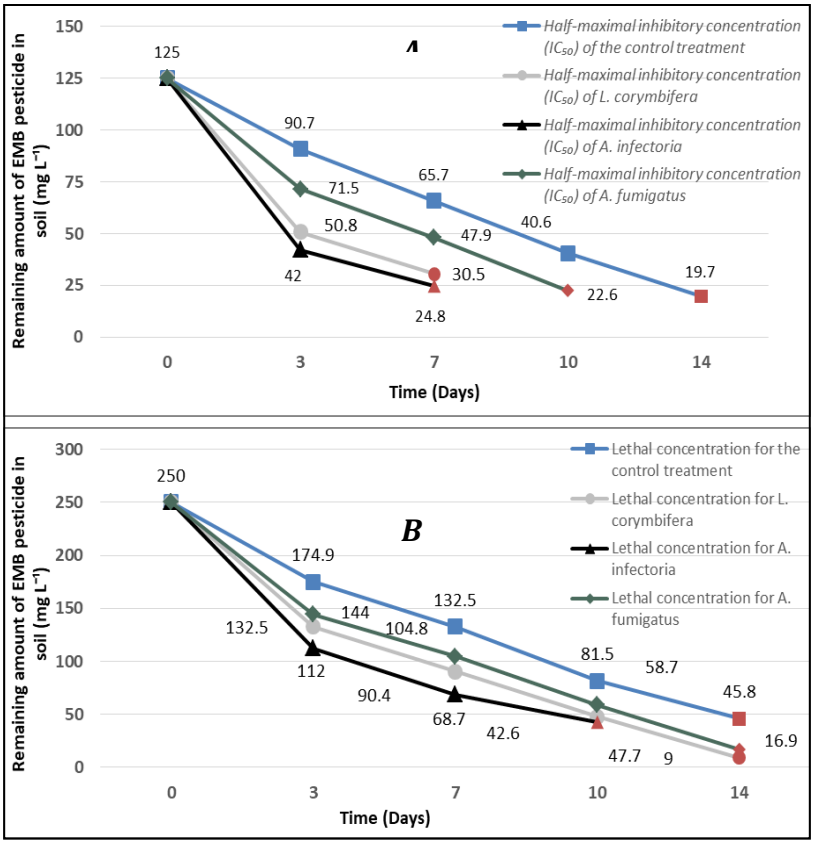

Figure (5) Kinetic behavior of the degradation of Emamectin Benzoate (EMB) residues in soil. (A), At the semi-lethal concentration (125 mg L^{-1}). (B), At the lethal concentration (250 mg L^{-1}).

Despite this severe inhibition, the curve of the *Alternaria infectoria* fungus emerged in (Figure 5B) as a unique biological exception. It maintained a rapid decline, recording in (Table 4) the highest kinetic efficiency at this elevated concentration k = 0.388 day^{-1} and a half-life $t_{1/2}$ = 1.79 days. This exceptional performance is explained by the *Alternaria* genus possessing advanced cellular defense mechanisms.

These include the production of melanin pigments in their cell walls to adsorb toxins, alongside active efflux pumps that expel pesticide molecules out of the cell. This physiological repertoire enabled the fungus to resist poisoning and continue secreting its degradative enzymes with high efficiency (Sharma *et al*., 2018). Consequently, based on these robust findings, the A. *infectoria* isolate stands out as a highly qualified bioremediation agent, exceptionally suited for the restoration of agricultural lands subjected to severe contamination by high doses of this pesticide.

(Figure 6) provides a comparative illustration of the half-life periods ($t_{1/2}$) of the pesticide residues in days. The chart demonstrates a pronounced reduction in the time required for pesticide degradation when utilizing bioremediation as opposed to natural degradation. Specifically, fungal inoculation successfully decreased the pesticide's persistence from 4.70–5.84 days in the uninoculated control treatments to less than two days at both the recommended and sub-lethal concentrations.

Furthermore, the data clearly indicate that the *Alternaria infectoria* isolate exhibited superior efficiency relative to the other evaluated isolates. Remarkably, it recorded the shortest half-life 1.79 days even under the severe chemical stress of the lethal concentration 250 mg L^{-1}. These compelling findings strongly advocate for the application of this specific isolate as a highly effective bioremediation agent, capable of significantly mitigating soil contamination caused by Emamectin benzoate residues in agricultural lands.

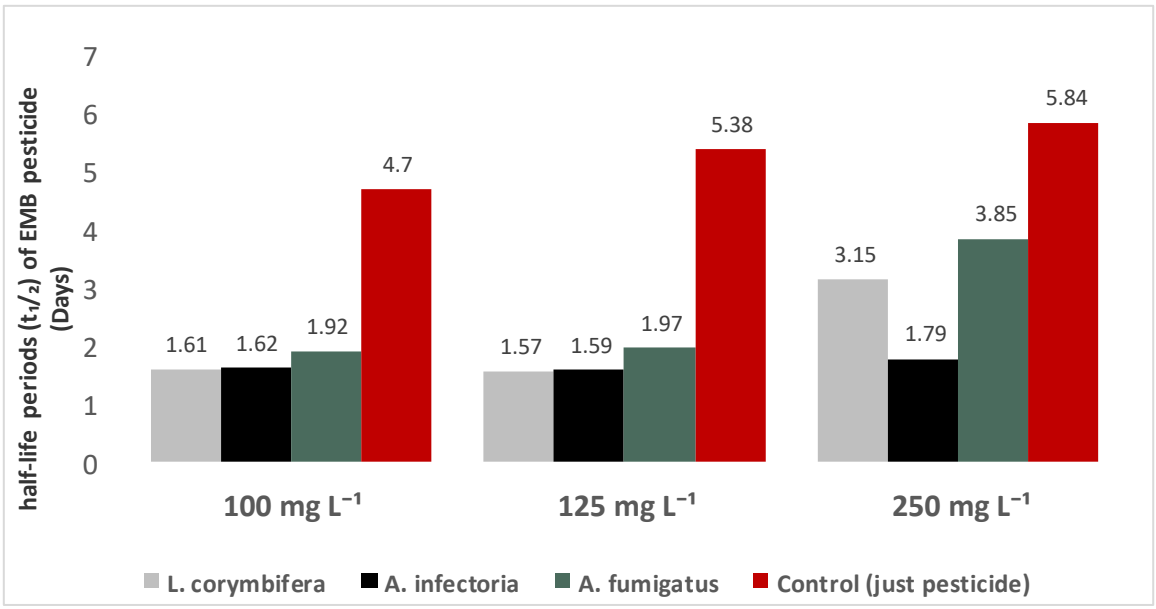

Figure (6) Comparison of the half-life periods ($t_{1/2}$) of EMB pesticide residues (days).

CONCLUSIONS

In this study, the aim was to assess the indigenous isolated fungi, *Alternaria infectoria*, *Lichtheimia corymbifera*, and *Aspergillus fumigatus* in degrading three different concentrations of EMB, involving 100, 125, and 250 mgL^{-1}. Furthermore, investigation of both soil respiration (CO_2 evolution) and urease activity. The outcomes revealed that the powerful capability to breakdown the EMB. This has been shown by increasing the level of CO_2, resulting in improved soil metabolic activity. the *A. infectoria* (F2) isolate showed remarkable superiority When using 250 mg L^{-1}, combining a quick reaction with cumulative tolerance. On the third day, this isolate showed an early respiratory surge, which was suggestive of increased enzymatic activity. It showed incredible tenacity, reaching a second recovery peak of soil on day 28, while going through a toxic shock and a subsequent drop on the seventh day. By the end of the incubation period, this active kinetic behavior resulted in a significant cumulative outcome, where the isolate achieved a high level of CO2 release and recorded the highest total CO2 accumulation at 250 mg L^{-1}.

Disclaimer: The authors declare that they have no relevant financial or non-financial interest to disclose.

Conflict of interest: The authors declare that they have no relevant financial or non-financial interest to disclose.

Funding disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The study was self-funded by the authors, with logistical and laboratory support provided by the College of Agriculture, University of Basrah.

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