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Research Article

Pathogenicity of *Metarhizium anisopliae* (Pacer) against Two Stored-Grain Insect Pests under Abiotic Stress Factors

[a]Awais Ali Chatha, [a]Muhammad Asrar, [b]Khuram Zia, [a]Usama Saleem, [c]Saba Malik, [a]Zeeshan Javed, [a]Hassan Usman, [d]Mubshar Saleem

[a] *Department of Zoology, Faculty of Life Sciences, Government College University, Faisalabad, Punjab, Pakistan.*
[b] *Office of Research, Innovation, and Commercialization, University of Agriculture, Faisalabad, Punjab, Pakistan.*
[c] *Agronomist, Engro Fertilizers Limited, Pakistan.*
[d] *Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Punjab, Pakistan.*

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ABSTRACT

Stored grains are attacked by numerous insect pests, among which *Rhyzopertha dominica* and *Trogoderma granarium* are the most significant. Extensive use of phosphine has led to the development of resistance in these pests, along with residual effects on stored grain commodities. In this study, a commercially available formulation of the entomopathogenic fungus *Metarhizium anisopliae* (Pacer) was evaluated through bioassays to determine the effects of temperature and relative humidity on its pathogenicity against *R. dominica* adults and *T. granarium* larvae. The bioassay results revealed that the efficacy of the fungal formulation was influenced by conidial dose rate, exposure period, temperature, and relative humidity. The highest mortality rates, 68.82% for *T. granarium* and 60.10% for *R. dominica*, were recorded at 30°C and 65% relative humidity with a dose rate of 9×10^8 conidia after a 21-day exposure. Relatively lower mortality values, 50% and 48%, were observed after 14 days of exposure, respectively. The 7-day exposure period combined with 55% relative humidity proved least effective, resulting in the lowest pathogenicity against both test insects. Lower dose rates of 3×10^8 and 6×10^8 conidia were also comparatively less effective against both species. These findings suggest that Pacer, an entomopathogenic fungal formulation, exhibits considerable entomocidal potential for managing stored-grain insect pests and could reduce reliance on phosphine and other chemical fumigants. However, the efficacy of entomopathogenic fungi in stored grain protection is strongly dependent on prevailing abiotic conditions.

Corresponding Author: Muhammad Asrar
Email: asrar@gcuf.edu.pk

Introduction

The presence of insect pests during storage poses serious risks to the safety and integrity of stored commodities (Phillips and Throne, 2010; Mason and Donough, 2012; Gerken and Morrison, 2022). Globally, nearly one-third of total food production becomes vulnerable to infestation by more than 20,000 insect pest species that attack both stored and field commodities. These infestations damage food during storage, while insect cadavers, frass, and exuviae further reduce the quality and marketability of stored products (Dubey et al., 2008). Grain products are attacked by hundreds of arthropod species, including approximately 600 beetle species (Coleoptera), 70 moth species (Lepidoptera), and about 355 mite species, all of which contribute to significant post-harvest losses (Rajendran and Sriranjini, 2008). Among these, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) and *Rhyzopertha dominica* (Fabricius) are regarded as the most destructive stored-grain pests worldwide (Plarre, 2010; Athanassiou et al., 2019).

Fumigants such as phosphine and insecticides like deltamethrin are widely used to protect stored grains from these pests. However, their extensive application has been associated with several drawbacks, including severe toxic effects on non-target organisms (Pisa et al., 2017; Rajendran, 2020), the development of insect resistance (Opit et al., 2012; Afful et al., 2017), pesticide residues in treated commodities (Lozowicka et al., 2014), and adverse impacts on the environment (Van der Sluijs et al., 2015). These concerns highlight the urgent need for innovative, environmentally sustainable alternatives for stored-grain protection, particularly in view of increasing consumer demand for organic products and the necessity of safeguarding vulnerable populations.

As an alternative to synthetic chemicals, entomopathogenic fungi (EPF) have been proposed for managing stored-grain pests (Batta and Kavallieratos, 2018; George et al., 2018; Mantzoukas et al., 2023). EPF are naturally occurring insect pathogens that exhibit low toxicity to mammals and negligible environmental impact (Sharma and Sharma, 2021). Extensive studies have confirmed their effectiveness against a range of stored-product insect pests (Rumbos and Athanassiou, 2017). Among these, *Metarhizium anisopliae* (Metschnikoff) (Batta, 2005; Michalaki et al., 2006) and *Beauveria bassiana* (Balsamo) (Padín et al., 2002; Kaur et al., 2014; Dal Bello et al., 2017; Rehman et al., 2019) are the most widely studied. Their infection process begins when asexual spores (conidia) attach to the insect cuticle. The germ tube then penetrates the integument, and the fungus proliferates internally, producing toxins that eventually kill the host (Pedrini et al., 2007). Subsequently, the mycelium emerges from the cadaver, sporulates, and enables secondary transmission to other insects (Gabarty et al., 2014).

The pathogenicity of EPF is strongly influenced by abiotic factors, particularly temperature and relative humidity (Sarwar, 2015). High temperatures can reduce conidial germination and viability (Horaczek and Viernstein, 2004), while the role of humidity has produced inconsistent results across studies (Akbar et al., 2004). Considering these abiotic influences and the challenges posed by climate change, the present study aimed to evaluate the efficacy of a commercially available strain of *M. anisopliae* against *R. dominica* and *T. granarium* under varying temperature and humidity regimes.

Materials and Methods

The current study was conducted at the Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture, Faisalabad, and at the Department of Zoology, Government College University, Faisalabad. The objective was to evaluate the effect of relative humidity (RH) and temperature on the efficacy of a commercially available formulation of the entomopathogenic fungus *M. anisopliae* against *R. dominica* and *T. granarium*.

Grains were cleaned and air-dried in the laboratory to attain a moisture content of approximately 12%. Prior to the experiment, seeds were surface sterilized to eliminate any pre-existing infestation. Samples of wheat grains (200 g each) were placed into 250 ml capacity plastic jars. The larvae of *T. granarium* and adults of *R. dominica* were obtained from stock cultures maintained under controlled laboratory conditions. Batches of 30 insects of each species were introduced separately into each jar. Treatments were arranged in a completely randomized design (CRD) with three replicates. All grains were stored under controlled growth conditions at 30 ± 2°C and 65 ± 5% RH.

Commodity

Bioassays were conducted on clean, untreated wheat grains free from insect infestation. The initial grain moisture content ranged from 10.1% to 12.2%, measured with a Dickey-John moisture meter (Multigrain CACII; Dickey-John Co., Auburn, IL, USA).

M. anisopliae formulation

A commercially available formulation (Pacer®) of *M. anisopliae* conidia was used, procured from AgriLifeTM (Plot No. 154/A5-1, IDA Bollaram-502325, Medak, Hyderabad District, Andhra Pradesh, India). The aqueous conidial suspension contained 1×10^8 conidia per gram.

Bioassay procedure

The insecticidal potential of Pacer® (*M. anisopliae*) was evaluated at three concentrations: 3×10^8, 6×10^8, and 9×10^8 conidia CFU/g, applied to the surface of 1 kg of wheat grains placed in cylindrical jars. Because of the powdery nature of the formulation, jars were manually shaken for approximately 10 min to ensure uniform distribution. From each treated batch, three 60 g subsamples were taken and placed in 250 ml plastic jars. Thirty larvae of *T. granarium* and thirty adults of *R. dominica* were introduced separately into the jars. Ventilation was maintained through fine perforated cloth fixed over small openings in the jar lids.

Untreated grains served as the control and were kept in a similar series of vials. All jars were placed in incubators (Model MIR-254, SANYO) set at different temperatures (26, 30, and 34°C) and relative humidities (55, 65, and 75%). For each treatment, a corresponding control was maintained. Each treatment was replicated three times. Insect mortality was recorded at 7, 14, and 21 days post-exposure.

Statistical analysis

Mortality data were corrected using Abbott's formula (Abbott, 1925). The pathogenicity data were subjected to three-way analysis of variance (ANOVA), with temperature, relative humidity, exposure time, and conidial dose as independent variables. Treatment means were separated using Tukey's HSD test at the 5% significance level (Sokal and Rohlf, 1995).

Results

Table 1 presents the ANOVA results for the effects of fungal treatments, concentrations, exposure periods, and relative humidity (RH) on the mortality of both insect pests. Significant dose-response interactions were observed with both temperature and RH ($P < 0.01$), indicating that the pathogenicity of *M. anisopliae* is influenced not only by spore concentration but also by its interactions with abiotic factors. Increasing conidial concentrations produced disproportionately higher mortality at 30°C compared with 25°C or 35°C, suggesting that fungal virulence is strongly modulated by temperature, with 30°C representing a near-optimal range for host-pathogen interactions.

Among the interaction effects, first-order interactions (Dose × Exposure period, Dose × Temperature, Dose × RH, Temperature × Exposure period, RH × Exposure period, RH × Temperature) were all highly significant ($P < 0.01$). Second-order interactions (Dose × RH × Temperature, Dose × RH × Exposure period) were also highly significant ($P < 0.01$), except for RH × Temperature × Exposure period and Dose × Temperature × Exposure period, which were not significant ($P > 0.05$). The third-order interaction was statistically significant ($P < 0.01$).

Table 1. ANOVA parameters for the pathogenicity of *M. anisopliae* against larvae of *T. granarium* and adults of *R. dominica.*

Source of Variation	DF	*T. granarium*		*R. dominica*	
		F-value	*P-value*	*F-value*	*P-value*
Temperature	2	606.2	<0.01	314.1	<0.01
Exposure	2	248.6	<0.01	920.2	<0.01
Dose rate	2	525.1	<0.01	698.0	<0.01
RH	2	260.3	<0.01	210.4	<0.01
Dose rates × Exposure	4	58.2	<0.01	134.4	<0.01
Dose rates × Temperature	4	18.3	<0.01	15.1	<0.01
Dose rates × RH	4	42.0	<0.01	54.6	<0.01
Temperature × Exposure	4	91.2	<0.01	79.7	<0.01
RH. × Exposure	4	98.5	<0.01	82.1	<0.01
RH × Temperature	4	112.4	<0.01	247.3	<0.01
Dose rates × RH× Temperature	8	6.2	<0.01	24.3	<0.01
Dose rates × RH. × Exposure	8	0.9	0.75	4.1	<0.01
Dose rates × RH × Temperature × Exposure	8	15.1	<0.01	40.1	<0.01
RH. × Temperature× Exposure	16	0.2	0.98	1.3	0.12
Dose rates ×Temperature× Exposure	8	0.8	0.80	1.8	0.11

Table 2 presents the variation in mean mortality of *R. dominica* at different conidial doses of *M. anisopliae*, temperatures, and RH levels across various exposure periods. The highest mortality (60.1%) was recorded at 9 × 10^8 conidia, 30°C, and 65% RH after 21 days of exposure, followed by 48.3% at 14 days and 36.1% at 7 days under the same conditions, indicating a clear dose- and time-dependent increase in pathogenicity. Other combinations of dose, temperature, and RH produced intermediate levels of pathogenicity against *R. dominica*. The lowest mortality (6.1%) was observed at 34°C and 75% RH after 7 days of exposure.

Table 3 shows the changes in mean mortality values of *T. granarium* at different conidial dose rates of *M. anisopliae*, RH levels, and temperatures after various exposure periods. The highest mortality (68.82%) was observed at a dose of 9 × 10^8 conidia, 30°C, and 65% RH after 21 days of treatment. Exposure periods of 14 and 7 days were comparatively less effective, resulting in mean mortality values of 50.5% and 38.12%, respectively, under the same dose rate, temperature, and RH. Intermediate mortality levels were recorded at other dose rates, temperatures, and RH conditions. The lowest mortality (8.10%) was observed at 55% RH and 34°C after an exposure period of 7 days.

Table 2. Pathogenicity (%) of adult *R. dominica* on wheat grains exposed to three dose rates of *M. anisopliae* conidial formulation over 7, 14, and 21 days under three relative humidity and temperature conditions.

Exposure (Days)	Temperature (°C)	RH (%)	Pathogenicity (%) ± SE		
			3 × 10^8	6 × 10^8	9 × 10^8
7	26	55	8.20 ± 0.20^{hi}	9.41 ± 0.14^{ij}	16.35 ± 0.22^{hi}
		65	18.10 ± 0.16^{ef}	26.42 ± 0.19^{fg}	33.02 ± 0.20^{de}
		75	12 ± 0.10^{fg}	17.10 ± 0.16^{fg}	22.50 ± 0.14^{de}
	30	55	10.05 ± 0.16^{g}	12.25 ± 0.10^{gh}	19.30 ± 0.18^{fg}
		65	25.41 ± 0.21^{cd}	30.02 ± 0.24^{e}	36.10 ± 0.25^{d}
		75	23.12 ± 0.15^{d}	26.17 ± 0.31^{fg}	31.40 ± 0.17^{e}
	34	55	7.60 ± 0.11^{hi}	8.90 ± 0.13^{ij}	14.21 ± 0.13^{i}
		65	15.30 ± 0.30^{f}	18.69 ± 0.16^{g}	22.60 ± 0.32^{g}
		75	6.12 ± 0.18^{i}	11.04 ± 0.14^{i}	13.20 ± 0.11^{i}
14	26	55	12.41 ± 0.15^{fg}	13.33 ±0.16^{i}	23.52 ± 0.21^{g}
		65	25.10 ± 0.13^{cd}	34.16 ± 0.13^{de}	47.10 ± 0.29^{c}
		75	17.35 ± 0.11^{jk}	25.83 ± 0.13^{fg}	36.23 ± 0.17^{d}
	30	55	18.67 ± 0.29^{ef}	21.16 ± 0.17^{g}	26.01 ± 0.19^{fg}
		65	39.21 ± 0.30^{b}	44.33 ± 0.17^{bc}	48.32 ± 0.12^{b}
		75	34.65 ± 0.27^{bc}	41.66 ± 0.16^{c}	46.50 ± 0.16^{c}
	34	55	8.02 ± 0.17^{hi}	12.50 ± 0.1^{i}	21.43 ± 0.24^{h}
		65	17.83 ± 0.22^{ef}	22.50 ± 0.16^{g}	29.16 ± 0.21^{e}
		75	15.45 ± $0.21l^{m}$	16.16 ± 0.15^{hi}	21.09 ± 0.25^{h}
21	26	55	17.14 ± 0.13^{ef}	25.30 ± 0.11^{fg}	29.60 ± 0.19^{e}
		65	43.12 ± 0.13^{ab}	47.53 ± 0.27^{ab}	54.04 ± 0.13^{ab}
		75	27.10 ± 0.31^{cd}	38.24 ± 0.2^{de}	43.36 ± 0.24^{cd}
	30	55	25.15 ± 0.22^{cd}	26.10 ± 0.16^{fg}	32.54 ± 0.21^{e}
		65	46.23 ± 0.16^{a}	50.43 ± 0.18^{a}	60.10 ± 0.24^{a}
		75	36.25 ± 0.19^{bc}	47.76 ± 0.24^{ab}	52.31 ± 0.21^{b}
	34	55	17.82 ± 0.11^{ef}	25.19 ± 0.13^{fg}	28.37 ± 0.26^{f}
		65	24.62 ± 0.12^{d}	33.40 ± 0.21^{e}	37.01 ± 0.13^{d}
		75	13.41 ± 0.12^{g}	22.18 ± 0.20^{g}	28.12 ± 0.19^{f}
F values			9.45	23.12	40.1
P values			<0.02	<0.01	<0.01

Treatment means followed by the same letter are not significantly different according to Tukey's HSD test (P = 0.05).

Table 3. Pathogenicity (%) of *T. granarium* on wheat grains treated with three dose rates of *M. anisopliae* conidial mixture under three relative humidity and temperature levels after 7, 14, and 21 days of exposure.

Exposure (Days)	Temperature (°C)	RH (%)	Pathogenicity % ± SE		
			3×10^8	6×10^8	9×10^8
		55	17.10 ± 0.04[fg]	21.30 ± 0.14[fgh]	26.10 ± 0.19[ef]
	26	65	25.04 ± 0.11[de]	28.60 ± 0.21[e]	32.16 ± 0.16[a]
		75	16.30 ± 0.10[fg]	20.78 ± 0.24[f]	27.43 ± 0.10[g]
		55	9.50 ± 0.04[hi]	15.30 ± 0.10[ghi]	18.40 ± 0.14[fgh]
7	30	65	29.10 ± 0.11[cde]	33.45 ± 0.12[d]	38.12 ± 0.20[ef]
		75	19.30 ± 0.15[f]	26.05 ± 0.12[e]	31.01 ± 0.21[f]
		55	8.10 ± 0.06[i]	13.56 ± 0.21[i]	16.92 ± 0.24[ij]
	34	65	12.34 ± 0.11[i]	15.10 ± 0.08[gh]	16.02 ± 0.10[fij]
		75	10.30 ± 0.08[i]	14.16 ± 0.10[gh]	15.20 ± 0.12[j]
		55	19.21 ± 0.11[f]	24.10 ±0.13[ef]	28.82 ± 0.24[g]
	26	65	33.02 ± 0.17[d]	39.21 ± 0.24 [cd]	45.57 ± 0.20[cd]
		75	22.10 ± 0.16[e]	30.10 ± 0.18[d]	42.30 ± 0.23[de]
		55	15.40 ± 0.16[gh]	18.33 ± 0.12[hij]	22.66 ± 0.13[f]
14	30	65	37.80 ± 0.13[b]	40.12 ± 0.27[c]	50.54 ± 0.29[cd]
		75	27.42 ± 0.19[de]	31.54 ± 0.25[d]	38.17 ± 0.12[ef]
		55	15.21 ± 0.13[gh]	18.10 ± 0.13[f]	24.70 ± 0.18[hi]
	34	65	20.61 ± 0.17[e]	23.30 ± 0.343[ef]	27.02 ± 0.15[g]
		75	13.79 ± 0.12[i]	15.40 ± 0.16[gh]	20.31 ± 0.14[i]
		55	24.41 ± 0.19[de]	25.30 ± 0.11[ef]	29.60 ± 0.19[g]
	26	65	48.10 ± 0.17[ab]	47.53 ± 0.27[abc]	58.04 ± 0.13[ab]
		75	29.35 ± 0.31[d]	38.24 ± 0.2 [cd]	41.36 ± 0.24[de]
		55	35.12 ± 0.26[c]	42.59 ± 0.16[bc]	32.54 ± 0.21[f]
21	30	65	50.05 ± 0.24[a]	60.14 ± 0.26[a]	68.82 ± 0.42[a]
		75	32.20 ± 0.27[cd]	49.15 ± 0.21[ab]	56.40 ± 0.28[b]
		55	17.82 ± 0.11[fg]	25.19 ± 0.13[ef]	28.37 ± 0.26[g]
	34	65	36.47 ± 0.12[c]	40.81 ± 0.31[c]	46.34 ± 0.24[cd]
		75	25.12 ± 0.17[de]	28.10 ± 0.23[de]	33.79 ± 0.20[f]
F values			7.62	11.35	15.1
P values			<0.01	<0.03	<0.01

Treatment means followed by the same letter are not significantly different according to Tukey's HSD test (P = 0.05).

Discussion

The effectiveness and pathogenicity of EPF for managing storage insect pests are strongly influenced by abiotic environmental factors, particularly relative humidity and temperature (Sarwar, 2015; Rumbos and Athanassiou, 2017). Temperature is widely regarded as a critical determinant of EPF efficacy against stored-grain insect pests. Several studies have demonstrated that rising temperatures reduce fungal germination and conidial viability (Moore et al., 2000; Horaczek and Viernstein, 2004). As noted by Thomas and Blanford (2003), temperature can profoundly affect an organism's susceptibility to infection.

In the present study, *M. anisopliae* exhibited the highest pathogenicity at 30°C against both target insect pests, with reduced efficacy at 25°C and 35°C. A key novel finding was the identification of strong dose-temperature and dose-humidity interactions. Athanassiou et al. (2017) similarly reported that *M. anisopliae* was more toxic to *E. kuehniella* larvae at 30°C than at 25°C. Likewise, Michalaki et al. (2006) showed that temperatures above 20°C enhanced *M. anisopliae* efficacy against *T. confusum* larvae. Our results align with these findings, indicating that pathogenicity increased from 25 to 30°C but declined at 35°C, suggesting an optimal temperature range of approximately 28-32°C.

The precise mechanisms underlying this pattern are not fully understood. Horaczek and Viernstein (2004) suggested that *M. anisopliae* exhibits mild heat tolerance, potentially linked to enhanced fungal metabolism and increased host activity at warmer temperatures (Fields and Korunic, 2000). However, the decline in pathogenicity between 30 and 35°C may reflect reduced conidial germination at elevated temperatures.

Similar temperature-dependent effects have been reported for other EPF. Athanassiou and Steenberg (2007) observed that *B. bassiana* pathogenicity against *S. granarius* adults declined when temperature increased from 25 to 30°C. Thompson and Reddy (2016) reported that *B. bassiana* efficacy against *T. castaneum* peaked at 25°C, while Latifian et al. (2018) found maximal pathogenicity of *B. bassiana* against *O. surinamensis* at 25°C compared with 15, 30, 35, and 40°C. In contrast, Shaheen et al. (2016) noted reduced *B. bassiana* efficacy against *C. chinensis* when temperature decreased from 30°C to 25°C. The discrepancy with our findings may reflect species-specific thermal sensitivity or strain-level variation among EPF (Klingen and Haugenand, 2006).

Taken together, these results suggest that *M. anisopliae* virulence is closely linked to its physiological requirements. Optimal performance at 30°C likely reflects a balance between increased fungal enzymatic activity (e.g., proteases, lipases, chitinases) facilitating cuticle degradation and heightened host metabolic activity, which enhances host-pathogen interactions. In contrast, temperatures above 32-35°C likely impose thermal stress on conidia, reducing membrane integrity, impairing enzyme activity, and lowering germination efficiency, ultimately diminishing infectivity.

Relative humidity also plays a decisive role in EPF infection dynamics. High humidity generally promotes conidial germination and fungal infection, whereas low humidity can constrain these processes. However, studies on stored-grain systems suggest more complex interactions. Navarro et al. (2012) noted that concerns about high humidity in cereal storage have limited EPF application. Some studies report no significant influence of humidity: for instance, Akbar et al. (2005) observed no difference in *B. bassiana* toxicity against *T. castaneum* larvae at 55% versus 75% RH, while Athanassiou et al. (2017) reported strain-specific differences in *M. anisopliae* efficacy against *E. kuehniella* at 55% and 75% RH, with effectiveness maintained at an intermediate level of 65% RH.

Interestingly, several studies indicate that lower RH may enhance EPF pathogenicity. Lord (2005, 2007a, 2007b), Michalaki et al. (2006), and Athanassiou and Steenberg (2007) all reported increased efficacy of *B. bassiana* or *M. anisopliae* at 55% RH compared with 75% RH. For example, *B. bassiana* was more effective against *R. dominica* and *T. castaneum* under drier conditions (Lord, 2005, 2007a), and against *S. granarius* at 55% versus 75% RH (Athanassiou and Steenberg, 2007). Michalaki et al. (2006) also found higher *M. anisopliae* pathogenicity against *T. confusum* larvae at 55% RH. Wakil et al. (2011) similarly reported greater *B. bassiana* efficacy against *R. dominica* at 55% than at 75% RH. In our study, *M. anisopliae* performed best at 55-65% RH, supporting the notion of an optimal humidity window for fungal performance.

The mechanisms remain unclear. Lord (2005) suggested that desiccation stress may alter insect cuticular chemistry, enhancing conidial adhesion, germination, and penetration, or may increase insect susceptibility to fungal infection. Thus, EPF may be particularly effective in storage environments characterized by dry conditions, such as warehouses. Application rates and exposure periods of conidia also critically affect EPF efficacy (Khashaveh et al., 2011; Rehman et al., 2019), highlighting the multifactorial nature of EPF performance.

Conclusions

Abiotic factors such as temperature and relative humidity significantly influence the efficacy of entomopathogenic fungi, particularly *Metarhizium anisopliae*. Maximum pathogenicity was observed at 30°C and 55-65% RH, whereas higher temperatures (≥ 35 °C) and suboptimal humidity reduced conidial viability and insect mortality. These findings emphasize the need to monitor and adjust storage conditions before and during Pacer® application. For practical implementation, Pacer® should be deployed under conditions within these optimal ranges; where this is not possible, integration with complementary strategies such as inert dusts, hermetic storage, or improved formulations is recommended.

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Authors' Contributions

AAC conducted the experiments and collected the data; MA and KZ conceived the idea, designed the study, and provided overall supervision; US, SM, and ZJ analysed the results and contributed to data interpretation; HU and MS critically reviewed and proofread the manuscript for clarity, accuracy, and consistency; All authors read and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

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