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Brief Report

Potential of *Aspergillus oryzae* XJ-1 for the Biological Control of Grasshoppers in Arid Grasslands of Northwestern China

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Abstract

More than 100 species of grasshoppers have been documented in the Xinjiang Uygur Autonomous Region of China, several of which cause serious damage to pasturelands and negatively affect animal husbandry. *Aspergillus oryzae* XJ-1, a fungal pathogen of locusts and grasshoppers, has previously shown pathogenic activity against locusts and grasshoppers in crop field, but its efficacy in natural grasslands has not yet been evaluated. To assess its potential for grasshopper management in grasslands, we conducted infection assays, field-cage experiments and field trials in Xinjiang, China, from 2024 to 2025. Infection assays indicated that *A. oryzae* XJ-1 could infect 7 grasshopper species: *Calliptamus italicus* (Itanlian locust), *C. barbarus*, *Oedipoda miniata*, *Oedaleus decorus*, *Sphingonotus coerulipes*, *Notostaurus albicornis*, and *Gomphocerus sibiricus*. In field-cage experiments, cumulative mortality rates of *O. miniata* and *C. barbarus* reached 73.3 ± 8.8% and 76.7 ± 6.7%, respectively 20 days after inoculation with *A. oryzae* XJ-1 at 10^6 conidia mL^{-1}. In field trials, grasshopper population reduction rates in treated plots reached 84.9 ± 4.3% and 59.7 ± 4.6% at 15 days after treatment with 3×10^{12} conidia ha^{-1} in Huocheng County in 2024 and 2025, respectively. In Bole County in 2025, the reduction rate reached 79.6 ± 4.8% at 5 days after treatment using the same dosage. These results suggested that *A. oryzae* XJ-1 has potential as a biological control agent against several grasshopper species in the grasslands of the Xinjiang region.

Keywords: grasshopper; *Aspergillus oryzae* XJ-1; biological control; reduction of population density; field trial

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1. Introduction

Locusts and grasshoppers are major agricultural pests worldwide, affecting crop production, grassland ecosystems, and rural livelihoods [1]. Under favorable environmental conditions, their populations can rapidly reach outbreak levels, causing severe economic and ecological damage [2]. Despite decades of research and large-scale efforts, locust and grasshopper outbreaks continue to represent a major challenge in many regions of the world.

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Since the 1940s, chemical insecticides, including organochlorines (dieldrin, chlordane, DDT, benzene hexachloride), organophosphates (malathion, fenitrothion), and pyrethroids (deltamethrin, cypermethrin), have been widely used for the control of locusts and grasshoppers [3,4]. Although, these compounds may leave persistent residues and negatively affect human health and non-target organisms [4]. Consequently, environmentally friendly biopesticides have been developed and widely used [5], including *Beauveria bassiana* [6,7], *Metarhizium* spp. [8,9], and *Nosema locustae* [10–12]. However, behavioral thermoregulation in locusts and grasshoppers may reduce the efficacy of fungal pathogens such as *Metarhizium* under field conditions, as infected insects can elevate their body temperature to inhibit fungal development [13–17].

Aspergillus oryzae is a filamentous fungus that has been widely used for millennia in the fermentation of traditional foods [18]. It is classified as Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration because it does not produce aflatoxins or other known carcinogenic metabolites [19–23]. Although some *Aspergillus* species are associated with mycotoxin production or opportunistic pathogenicity, *A. oryzae* has long been recognized as a safe industrial microorganism and is extensively used in food fermentation. Species of *Aspergillus* have rarely been investigated as microbial control agents against insects. However, previous field-cage experiments and large-scale field trials conducted in Kenli County, Shandong Province, China, have shown that *A. oryzae* XJ-1, a fungal isolate originally obtained from a dead grasshopper collected in Xinjiang, exhibits pathogenic activity against several locust and grasshopper species, affecting both nymphal and adult stages in croplands [24–26]. Nevertheless, its efficacy against grasshopper populations in natural grasslands has not yet been evaluated.

More than 100 species of locusts and grasshoppers occur in the 57 million hectares of grassland in the Xinjiang region of northwestern China [27,28]. Several species periodically cause severe damage to grasslands and negatively affect animal husbandry. Current management strategies include chemical insecticides [29], *N. locustae* [30], *Metarhizium flavoviride* [31], and conservation of migratory birds such as *Sturnus roseus* [32]. However, few studies have evaluated microbial agents against adult grasshoppers in these arid grassland ecosystems. Because *A. oryzae* XJ-1 was originally isolated from a grasshopper collected in this region, we hypothesized that it may have potential as a biological control agent against grasshopper populations in the grasslands of Xinjiang. Therefore, we conducted infection assays, field-cage experiments, and field trials to evaluate its pathogenicity and field efficacy against grasshopper populations.

2. Materials and Methods

2.1. Field-Cage Bioassay of the Virulence of A. oryzae XJ-1 Against Adult Grasshoppers

Conidial powder of *A. oryzae* XJ-1 was prepared by the Biological Control Laboratory, Institute of Plant Protection, Shandong Academy of Agricultural Sciences. The conidial powder was suspended in 0.3% (v/v) Tween-80 solution at a concentration of 10^6 conidia mL^{-1}, and conidial density was determined using a hemocytometer. Adult *Oedipoda miniata* (Pallas, 1771) and *Calliptamus barbarus* (Costa, 1836) were collected from grasslands in Huocheng County, Yili Kazak Autonomous Prefecture, Xinjiang Uygur Autonomous Region, China. Grasshoppers were maintained outdoors in cages and fed fresh alfalfa seedings daily. Enclosures were cleaned regularly and feces removed daily.

The field-cage experiment was conducted at the Yili Prefecture Desert Steppe Experimental Station in Huocheng County. Sixty adults of each species were randomly assigned to six groups, including three treatment groups and three control groups, with 10 insects per group. Grasshoppers in the treatment groups were inoculated with *A. oryzae* XJ-1 at a concentration of 10^6 conidia mL^{-1}, whereas control insects were treated only with 0.3% (v/v) Tween-80 solution. For inoculation, each adult grasshopper was individually immersed in the conidial suspension for less than 1 s. After treatment, insects were air-dried and individually transferred to plastic boxes (top diameter: 14 cm; bottom diameter: 9 cm; height: 14 cm). Control insects were treated similarly using Tween-80 solution only. All plastic boxes were placed outdoors on the ground under natural environmental conditions.

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The experiment was conducted from 20 August to 8 September 2024, during which ambient temperatures ranged from 8 to 32 °C. Mortality was recorded daily for 20 days after treatment.

2.2. Field Trial Plots

Treatment Plot 1 (44°07′17.7″N, 80°55′54.3″E; altitude 723.3 m), covering 6.67 ha, was established in grassland of Huocheng County. An untreated control plot (CK 1) of the same size was located 100 m from Plot 1. The grasshopper community in Plot 1 consisted mainly of six species: *Calliptamus italicus* (Linnaeus, 1758), *O. miniata*, *C. barbarus*, *Oedaleus decorus* (Germar, 1825), *Sphingonotus coerulipes* (Uvarov, 1922), and *Notostaurus albicornis* (Eversmann, 1848). At the time of treatment, more than 95% of individuals were adults. The dominant vegetation in Plot 1 and CK 1 consisted of *Artemisia capillaris*, *Medicago falcata*, and *Sophora alopecuroides*.

Treatment Plot 2 (44°07′32.5″N, 81°00′45.5″E; altitude 793.6 m), covering 33.35 ha, was also established in grassland of Huocheng County, where adult *C. italicus* was the dominant grasshopper species. An untreated control plot (CK 2) of the same size was located 200 m from Plot 2. The dominant vegetation consisted mainly of *A. capillaris*.

Treatment Plot 3 (44°49′36″N, 81°39′59.0″E; altitude 2292.98 m), covering 33.35 ha, was established in grassland of Bole County, where adult *Gomphocerus sibiricus* (Linnaeus, 1767) was the dominant species. An untreated control plot (CK 3) of the same size was located 200 m from Plot 3. The dominant vegetation consisted of *Artemisia frigida*, *A. capillaris* and *M. falcata*.

No significant rainfall occurred during the three field trials.

An aqueous suspension of *A. oryzae* XJ-1 conidia at a concentration of 2×10^8 conidia mL^{-1} was applied by aerial spraying using a T40 agricultural drone (DJI, Shenzhen, China) at an application rate of 3×10^{12} conidia ha^{-1} in all treatment plots. Plot 1 and Plot 2 were treated in August of 2024 and July 2025, respectively, whereas Plot 3 was treated in July of 2025.

Grasshopper densities were surveyed one day before treatment and on days 5 (or 6), 10, and 15 after treatment. Sampling was conducted using a 1-m^2 quadrat (plastic frame fitted with nylon mesh), placed every 30 steps along a diagonal transect across each plot. Fifty samples were collected from each treatment and control plot during each survey, and the number of grasshoppers within each quadrat was recorded. Population reduction rates were calculated according to the method described by You [25].

Dead grasshoppers were collected individually from treatment plots to assess fungal colonization. Each cadaver was placed on a sterilized glass slide over sterilized filter paper in a sterile Petri dish. To maintain high humidity, 200 μL of sterile water was added to the filter paper. Petri dishes were incubated at approximately 28 °C for 7 days and examined daily. The presence of *A. oryzae* XJ-1 mycelial growth on the insect surface was considered indicative of successful fungal colonization.

2.3. Statistical Analysis

All statistical analyses were performed using Origin 8.0 software (OriginLab, Northampton, MA, USA). Student's t-tests were used to compare cumulative mortality between treatment and control groups for adult *O. miniata* and *C. barbarus* in the field-cage bioassay. The same approach was used to compare grasshopper population densities and population reduction rates between treatment and control plots in each field trial. Statistical significance was determined at $p < 0.05$.

3. Results

3.1. The Virulence of *A. oryzae* XJ-1 Against Adult Grasshoppers in the Field-Cage Experiment

After being inoculated with *A. oryzae* XJ-1 in the field-cage experiment, adult grasshoppers *O. miniata* and *C. barbarus* began to die on the second or third day, with mortality gradually increasing thereafter (Figure 1). By day 20, mortality in treated *O. miniata* was 73.3 ± 8.8%, and that of *C. barbarus* reached 76.7 ± 6.7%, whereas mortalities in the control groups were 26.7 ± 12.0% and 30.0 ± 11.5%,

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respectively. These results indicate that *A. oryzae* XJ-1 is pathogenic to adult *O. miniata* and *C. barbarus*, and suggest its potential as a biological control agent against these species.

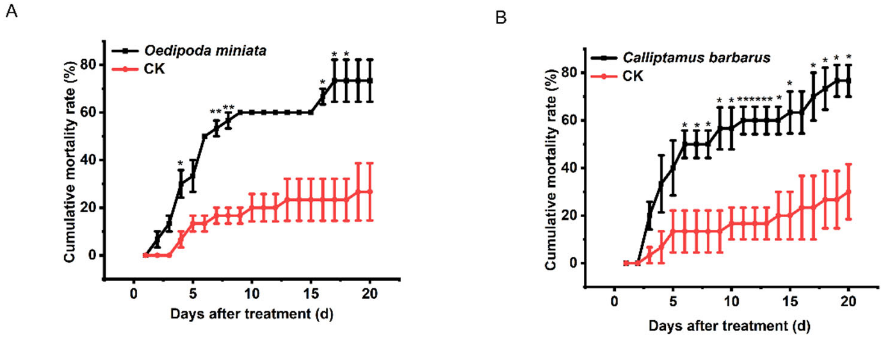

Figure 1. Cumulative mortality of adult grasshoppers exposed to *A. oryzae* XJ-1 in a field cage experiment. *O. miniata* (A) and *C. barbarus* (B). Inoculation concentration: 10^6 conidia mL^{-1}. Bars represent SE. $p < 0.05$ (*), $p < 0.01$ (**). Student's *t*-test (n = 3).

3.2. The Efficacy of A. oryzae XJ-1 Against Adult Grasshoppers in the Field

In Plot 1, the mean grasshopper density decreased from one day before treatment to 15 days after the application of *A. oryzae* XJ-1 conidia in 2024 (Table 1). No significant differences in mean grasshopper density were observed between the treatment and control plots one day before treatment ($p > 0.05$), or at day 6 ($p > 0.05$) and day 10 ($p > 0.05$) after treatment. However, the mean grasshopper density in the treatment plot was significantly lower than that in the control plot on day 15 (p < 0.001) after treatment (Table 1).

In Plot 2 (2025), a similar trend was observed, with a decrease in mean grasshopper density from one day before treatment to 15 days after application of *A. oryzae* XJ-1 conidia (Table 1). No significant differences were detected between the treatment and control plots one day before treatment ($p > 0.05$), nor at day 5 ($p > 0.05$) or day 10 ($p > 0.05$) after treatment. However, a significant difference in mean grasshopper density was observed at day 15 after treatment ($p < 0.001$) (Table 1).

In 2024, reduction rates in the treatment plot were 54.8 ± 5.2% (day 10) and 84.9 ± 4.3% (day 15), both significantly higher than those in the control plot on the corresponding dates: 24.9 ± 8.7% ($p < 0.05$) and 52.2 ± 7.8%, respectively ($p < 0.05$ for both comparisons; Figure 2). No significant difference in reduction rates was observed between the treatment (6.0 ± 14.7 %) and the control plots (-15.6 ± 35.0 %) at day 6 ($p > 0.05$) (Figure 2).

In 2025, reduction rates in the treatment plot were 14.3 ± 0.8% (day 5), 43.3 ± 4.2% (day 10), and 59.7 ± 4.6% (day 15), all significantly higher than those in the control plot at the corresponding times: -13.7 ± 9.8% ($p < 0.05$), 9.8 ± 1.1% ($p < 0.01$), and 2.6 ± 0.6% ($p < 0.001$), respectively.

Table 1. Mean grasshopper density (individuals m^{-2}) in treatment and control plots (CKs) in Huocheng County, China, in 2024 and 2025.

2024			2025		
Survey day	CK 1 (mean ± SE)	Plot 1 (mean ± SE)	Survey day	CK 2 (mean ± SE)	Plot 2 (mean ± SE)
1 day before treatment	1.76 ± 0.28a	1.82 ± 0.21a	1 day before treatment	3.92 ± 0.50a	5.06 ± 0.54a
6 days	1.84 ± 0.41a	1.68 ± 0.34a	5 days	4.44 ± 0.41a	4.34 ± 0.52a
10 days	1.28 ± 0.23a	0.84 ± 0.16a	10 days	3.54 ± 0.37a	2.88 ± 0.40a
15 days	0.80 ± 0.13a	0.26 ± 0.08b	15 days	3.82 ± 0.38a	2.02 ± 0.32b

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Note: Different letters following the values indicate significant differences between treatment and control plots (CK) on the same day within the same trial (*t*-test).

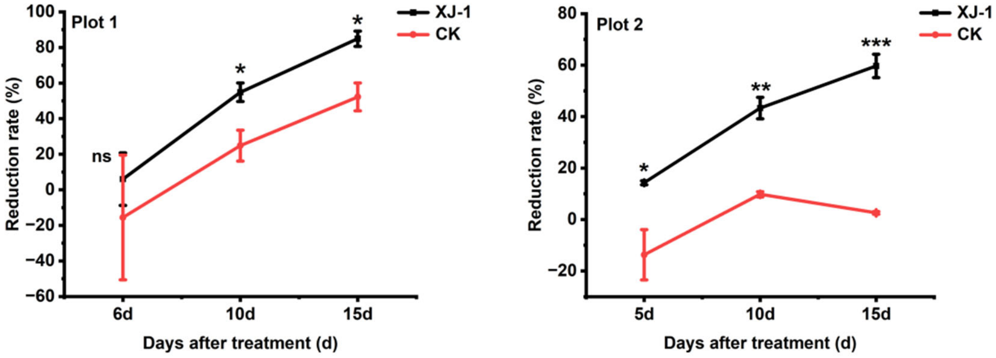

Figure 2. Reduction rate of adult grasshoppers after treatment with *A. oryzae* XJ-1 in field trials in Huocheng County, China. Plot 1 was treated in 2024, and Plot 2 in 2025. Asterisks indicate significant differences between treatment and control on the same day, according to Student's *t*-tests. ns, not significant. $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). Bar represent SE.

In Plot 3, mean grasshopper population density significantly declined over time in both the treatment and control plots from one day before treatment to 15 days after aerial application of *A. oryzae* XJ-1 conidia (Table 2). The mean grasshopper density in the treatment plot was significantly lower than that in the control plot at day 5 ($p < 0.001$), day 10 ($p < 0.001$), and day 15 ($p < 0.01$) after treatment (Table 2). Reduction rates in the treatment plot were 79.6 ± 4.8% (day 5) and 93.2 ± 1.6% (day 15), both significantly higher than those in the control plot on the corresponding days: 46.6 ± 4.5% ($p < 0.01$) and 79.4 ± 4.0% ($p < 0.05$), respectively (Figure 3). No significant difference in reduction rate was observed at day 10 between the treatment (89.7 ± 2.1%) and control plots (54.4 ± 13.7%) ($p > 0.05$) (Figure 3).

Table 2. Mean grasshopper density (individuals m^{-2}) in treatment Plot 3 and control Plot 3 (CK3) in Bole County, China, in 2025.

Survey day	CK 3 (mean ± SE)	Plot 3 (mean ± SE)
1 day before treatment	4.00 ± 0.32a	4.00 ± 0.32a
5 days	2.16 ± 0.21a	0.80 ± 0.14b
10 days	1.75 ± 0.24a	0.40 ± 0.09b
15 days	0.80 ± 0.13a	0.28 ± 0.09b

Note: Different letters following the values indicate significant differences between plots on the same day according to Student's *t*-test.

Mycelia growth of *A. oryzae* XJ-1 was observed on the bodies of seven grasshopper species—*O. miniata*, *C. barbarus*, *S. coerulipes*, *O. decorus*, *C. italicus*, *N. albicornis*, and *G. sibiricus*—collected from the treatment plots after 4–5 days of incubation. This indicates that *A. oryzae* XJ-1 infected and killed these seven species under field conditions after application (Figure 4).

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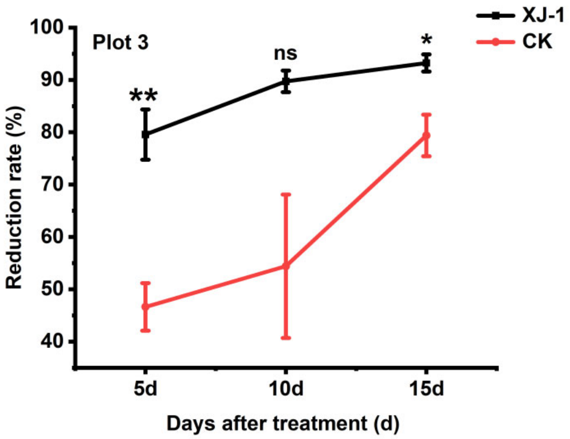

Figure 3. Reduction rate of adult grasshoppers after treatment with *A. oryzae* XJ-1 in a field trial in Bole City, China, in 2025. Asterisks indicate significant differences between the treatment and control on the same day according to Student's *t*-tests. ns, not significant. $p < 0.05$ (*); $p < 0.01$ (**). Bar represent SE.

Figure 4. External fungal growth and sporulation of *A. oryzae* XJ-1 on the bodies of seven species of dead adult grasshoppers collected in the treatment plot. (A) *Oedipoda miniata*; (B) *Calliptamus barbarus*; (C) *Sphingonotus coerulipes*; (D) *Oedaleus decorus*; (E) *Calliptamus italicus*; (F) *Notostaurus albicornis*; (G) *Gomphocerus sibiricus*.

4. Discussion

The efficacy of biocontrol agents is strongly influenced by several characteristics of the grasslands in Xinjiang. These grasslands are characterized by: (1) drought stress, with annual rainfall often below 300 mm; (2) stronger ultraviolet radiation, which may impair conidial viability and delay germination [33]; and (3) high grasshopper diversity, with more than 100 species occurring from early to late in the growing season [27].

Metarhizium spp. and *N. locustae* have previously been used for grasshopper control in the Xinjiang region. The corrected reduction rate of a *Metarhizium* strain against mixed nymph and adult *C. barbarus*, *N. albicornis*, *O. decorus*, *Sphingonotus* spp., and *Dociostaurus* spp. was only 37.60 ± 2.99% 14 days after treatment [34]. Field control of mixed adult *G. sibiricus*, *Stenobothrus eurasius*, *Stauroderus scalaris*, *Chorthippus albomarginatus*, *Dociostaurus kraussi* in Xinjiang using an oil formulation of *Metarhizium flavoviride* at the dose of 5 × 10^{12} conidia / ha resulted in a corrected mortality rate of

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60.9% after 16 days [31]. Application of the microsporidium *N. locustae* at concentrations of 100-150 million spores per 100 g of wheat bran produced corrected mortality rates of only 36.1% to 56.2% against 2nd – 3rd instar *C. italicus* after 28 days [30].

Our field results suggest that *A. oryzae* XJ-1 may be better adapted to the environmental conditions of Xinjiang grasslands, as it originates from this region and may therefore exhibit enhanced performance and higher mortality rates under local field conditions. These findings suggest that *A. oryzae* XJ-1 has strong potential for grasshopper control in arid grasslands, although additional field trials are still needed. No adverse effects on non-target organisms were evaluated in the present study and should be investigated in future work.

The reduction rates of CK 1 and CK 3 are quite high in the field trials. The possible reason may be that the grasshoppers in the plots migrate to other places. *C. italicus* and *G. sibiricus* in Xinjiang, particularly for adults, both have strong migratory ability [1,35]. *C. italicus* nymph could migrate more than 400 m/d [1]. *G. sibiricus* adult could migrate several hundred meters one time [35]. This may affect the control efficacy when investigated. To reduce the effect of migration, the area of field trial should be enlarged to several hundred hectares.

Previously, we demonstrated that *A. oryzae* XJ-1 was effective against adults of *Locusta migratoria*, *Epacromius* spp., *Atractomorpha* spp., and *Oxya* spp. in a large-scale field experiment conducted in Kenli County, Shandong Province, China, in 2022 under temperatures ranging from 26 to 38 ℃ [25]. During the present three field trials, temperatures ranged from 6 to 37 ℃. These findings suggest that the effective temperature range of *A. oryzae* XJ-1 against adult locusts and grasshoppers in the field is broad, spanning from 6 to 38 ℃, which could considerably widen its potential application.

However, it remains unknown whether infected grasshoppers can behaviorally increase their body temperature, as reported for infection by *Metarhizium acridum*, thereby reducing infection success and control efficacy [13–17,36]. This question deserves further investigation.

Here, we demonstrated that *A. oryzae* XJ-1 is also effective against adult *O. miniata*, *C. barbarus*, *S. coerulipes*, *O. decorus*, *C. italicus*, *N. albicornis* and *G. sibiricus* under field conditions. Hight virulence against adult locusts and grasshoppers appears to be a distinguishing characteristic of *A. oryzae* XJ-1.

Specific virulence mechanisms may be involved in its pathogenicity toward adult locusts and grasshoppers. For example, *Metarhizium* spp. produce destruxins that contribute to locust mortality [37], whereas *B. bassiana* produces several insecticidal metabolites, including beauvericin, bassianolide, beauverolide, bassianin, tenellin, oosporein, oxalic acid, and calcium oxalate crystals, with beauvericin considered one of the major toxins involved in pathogenicity [38,39]. Further studies are therefore needed to elucidate the pathogenic mechanisms of *A. oryzae* XJ-1 against locusts and grasshoppers.

Author Contributions: Y.Y., Y.S. and M. L. conceived and designed research. J.D., X.X., F.Y. and Y.N. conducted the experiments; Y.Y., Y.S. and M. L. wrote the manuscript. All authors read and approved the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries may be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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