

Investigation of In Vitro Antifungal Activity of Mistletoe (*Viscum album* ssp.) Living on Almond Tree (*Prunus dulcis* sp.)

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ABSTRACT

Mistletoe lives as a semi-parasite on the almond tree (*Prunus dulcis*) within the borders of Hacılar district of Kayseri province; by obtaining leaf plant extracts of (*Viscum album* ssp. *album*), *Fusarium moniliforme* and *Fusarium oxysporum* f. sp. Control and experimental groups were prepared to investigate the antifungal activity against *melongenae*. Dose-1 extracts prepared as 10mg/ml and Dose-2 extracts prepared as 20mg/ml were applied. Two application doses and a control group were applied for fungal pathogens in 6 replicates. In the control group, sterile distilled water was added. Mixed potato dextrose agar (PDA) containing plant extract was prepared as media. According to the Tukey/Kramer Test, different doses of *V. album* ssp. *album* extract were found to detect *F. oxysporum* f. sp. The effect on the development of *Fusarium melongenae* and *Fusarium moniliforme* was compared. Dose-1 and Dose-2 have a significantly higher *F. oxysporum*, f. sp. *F. melongenae*, and *Fusarium moniliforme* effect on colony development than the control. It was also examined whether there was a difference in effect between the two fungi, and it was observed that *V. album* ssp. *album* extract had similar effects on both fungal species. Statistical results ($p < 0001$) were significant at both doses. In conclusion, It was determined that mistletoe leaf extracts showed antifungal activity against both fungal species at Dose-1 and Dose-2 rates.

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INTRODUCTION

Mistletoe (*Viscum* L.) is a well-known semi-parasitic plant from the *Viscaceae* family, with widespread distribution and diverse ecological importance. It thrives by attaching itself to the shoots, branches, and trunks of host trees, extracting water and nutrients through specialized structures known as haustoria while maintaining its ability to photosynthesize (Üstüner & Aksoy, 2021). This unique combination of parasitism and photosynthesis makes mistletoe an intriguing subject for ecological, pharmacological, and agricultural studies. Among the most common genera within the group are *Viscum*, *Arceuthobium*, and *Loranthus*, which parasitize various host species, including fir, almond, maple, and oak (Kienle et al., 2011).

The species *Viscum album* L., commonly known as European mistletoe, has drawn attention for its dual role as a plant parasite and its therapeutic applications. It is known to affect diverse host trees in Türkiye, with varying levels of prevalence and impact. For instance, a study in Nigde,

Türkiye, recorded a high prevalence of *Viscum album* on almond (*Amygdalus* spp.) trees, with infection rates reaching 48.54%, highlighting its potential to cause significant agricultural damage (Üstüner, 2022). These findings underscore the need for a deeper understanding of mistletoe's interactions with its hosts, particularly in agricultural settings.

Mistletoe has been used in traditional medicine for centuries across cultures. Historically, it has been employed to treat labor pains, gout, liver ailments, and hypertension (Büssing, 2000; Gürhan & Ezer, 2004). Rudolf Steiner first proposed its use in cancer treatment in 1920, hypothesizing that mistletoe extracts might possess anticarcinogenic properties (Steiner, 1985). Subsequent studies have confirmed the therapeutic potential of *Viscum album*, with extracts demonstrating immunomodulatory and tumor-inhibitory effects in vitro and in clinical settings (Szurpnicka et al., 2020; Baek et al., 2021).

Recent advancements in phytotherapy have highlighted mistletoe's antimicrobial properties. For example, extracts of *Viscum album* have shown inhibitory effects against several

pathogenic microorganisms, emphasizing its potential as a natural antimicrobial agent (Ostermann et al., 2009). These findings align with the broader interest in exploring plant-based solutions to combat microbial resistance and reduce reliance on synthetic chemicals.

In addition to its therapeutic applications, mistletoe's potential as an antifungal agent has garnered attention. *Fusarium* species, particularly *Fusarium moniliforme* and *Fusarium oxysporum*, are major plant pathogens that cause significant economic losses in agriculture (Parihar et al., 2022). The indiscriminate use of synthetic fungicides to combat these pathogens has led to environmental degradation, soil fatigue, and health concerns (Bricarello et al., 2023). Consequently, there is a pressing need for eco-friendly alternatives, and mistletoe extracts present a promising solution.

Previous studies have demonstrated the antifungal properties of essential oils and plant extracts, such as those from *Artemisia*, *Cuminum cyminum*, and *Allium sativum* (Kordali et al., 2005; Peng et al., 2021). However, the antifungal potential of *Viscum album* remains underexplored. Early investigations suggest that mistletoe extracts could inhibit the growth of certain fungal pathogens, but comprehensive studies focusing on specific *Fusarium* species are lacking (Rizwan et al., 2021).

Despite its diverse applications, several gaps persist in the understanding of *Viscum album*'s antifungal potential. While its traditional uses and anticarcinogenic properties have been extensively studied, research on its role in agricultural pest management remains limited. Specifically, there is insufficient data on the efficacy of mistletoe extracts against *Fusarium* species, which are among the most challenging plant pathogens.

This study aims to address existing gaps in the literature by investigating the antifungal activity of *Viscum album ssp. album* extracts derived from almond trees (*Prunus dulcis*) against two significant fungal pathogens, *Fusarium moniliforme* and *Fusarium oxysporum f. sp. melongenae*. The research focuses on evaluating the inhibitory effects of different concentrations of mistletoe extracts to provide a scientific foundation for their application in sustainable agriculture. Specifically, the objectives of this study are to assess the antifungal efficacy of *Viscum album ssp. album* extracts against these *Fusarium* species *in vitro*, to determine the dose-dependent effects of the extracts on fungal growth inhibition, and to compare the sensitivity of the two fungal species to the extracts, thereby identifying potential variations in their susceptibility.

This study contributes to the growing body of research on plant-based antifungal agents by providing novel insights into the bioactivity of *Viscum album*. Unlike previous studies that focused primarily on mistletoe's medicinal properties, this research emphasizes its agricultural applications, particularly in pest and disease management. By demonstrating its antifungal potential, this study lays the groundwork for integrating mistletoe extracts into sustainable agricultural practices, reducing reliance on synthetic fungicides.

Furthermore, this research aligns with global efforts to promote eco-friendly pest management strategies. By highlighting the dual benefits of mistletoe—its role as a therapeutic agent and its potential as a natural fungicide—this study underscores the need for interdisciplinary approaches to address agricultural and environmental challenges.

The central hypothesis of this study is that *Viscum album ssp. album* extracts possess significant antifungal activity

against *Fusarium moniliforme* and *Fusarium oxysporum f. sp. melongenae*. It is expected that higher extract concentrations will exhibit greater inhibitory effects, consistent with findings from similar studies on plant-based antifungal agents (Kordali et al., 2005; Gilles et al., 2010). Additionally, variations in the susceptibility of the two *Fusarium* species to mistletoe extracts are anticipated, providing insights into the specificity of its antifungal properties.

In summary, this study seeks to bridge critical knowledge gaps by exploring the antifungal potential of *Viscum album ssp. album*. By addressing the challenges posed by *Fusarium* species, this research contributes to the development of sustainable agricultural practices and expands the applications of mistletoe beyond its traditional and medicinal uses. The findings are expected to provide a foundation for future studies on the mechanisms underlying mistletoe's antifungal activity and its practical applications in agriculture.

METHOD

Plant and Fungus Materials

This study utilized mistletoe (*Viscum album ssp. album*) collected from almond trees (*Prunus dulcis*) in the Hacılar district of Kayseri, Türkiye. The plant material was carefully separated from its host tree trunks and transported to the laboratory under controlled conditions to ensure its integrity. The fungal pathogens used in the study, *Fusarium moniliforme* and *Fusarium oxysporum f. sp. melongenae*, were sourced from the culture collection of the Mycology Laboratory at the Faculty of Agriculture, Erciyes University. The experimental setup included six replicates for each fungal species and three groups: two treatment groups with different extract doses and a control group.

Preparation of Extracts and Determination of Antifungal Effect

The leaves of mistletoe were dried under shade in a well-ventilated environment for 14 days to preserve bioactive compounds. After drying, the leaves were powdered using a blender and divided into two extract concentrations: Dose-1 (10 mg/mL) and Dose-2 (20 mg/mL). The powdered leaves were mixed with distilled water and stored in tightly sealed bottles at 4°C for 48 hours to allow complete extraction. The mixtures were then filtered through double-layered cheesecloth to remove debris, and the filtrates were used to prepare the treatments (Kordali et al., 2005).

Potato dextrose agar (PDA) was prepared by dissolving 3 g of PDA powder into the mistletoe extract and sterilizing the mixture in an autoclave at 121°C for 20 minutes. Control PDA plates were prepared similarly, using sterile distilled water instead of mistletoe extract. Each Petri dish (9 cm diameter) was poured with 20 mL of the prepared media under a laminar-flow hood and allowed to solidify. The plates were inoculated with 5 mm mycelial plugs of the target fungi and incubated in a dark environment at 24°C.

Measurement of Fungal Growth

The colony diameters of *Fusarium moniliforme* and *Fusarium oxysporum f. sp. melongenae* were measured every 48 hours for a total incubation period of 10 days.

Measurements were conducted using a digital caliper to ensure precision. To account for variations in growth patterns, the colony diameter was recorded at two perpendicular points, and the mean value was calculated. Environmental factors such as temperature, humidity, and light exposure were carefully controlled throughout the experiment to maintain consistent conditions.

The dark incubation at 24°C was chosen to simulate optimal fungal growth conditions while preventing external variables such as light-induced stress from influencing the results (Peng et al., 2021). The chosen doses of mistletoe extract (10 mg/mL and 20 mg/mL) were based on prior studies demonstrating significant bioactivity at similar concentrations (Kordali et al., 2005; Gilles et al., 2010). The use of PDA as a growth medium ensured a consistent nutrient supply for the fungi, minimizing variability in experimental outcomes.

Statistical Analysis

The antifungal efficacy of mistletoe extracts was evaluated using one-way Analysis of Variance (ANOVA). The differences in fungal growth among the three groups (Dose-1, Dose-2, and control) were assessed using Tukey's HSD post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$. The analysis was performed separately for each fungal species to identify species-specific responses to the treatments.

RESULTS OF STUDY

The study examined fungal growth in Petri dishes containing plant extract and nutrient medium to determine whether it differed from that in control Petri dishes without plant extract (Figure 1 and Figure 2). The effect of Dose-1 and Dose-2 on *F. Moniliforme* colony development is considerably greater than that of the control dose. At the same time, the effects of Dose-1 and Dose-2 on colony development are quite close. However, Dose-2 showed the most effective antifungal activity (Figure 1).

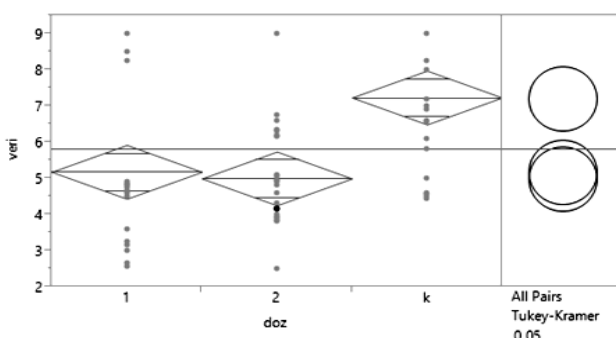


Figure 1. Comparison of the effect of different doses of *Viscum album ssp.* extract on *F. moniliforme* colony development (applications, 1: 10mg/ml; 2: 20mg/ml; k: Control).

Table 1. Statistical results showing the effect of *Viscum album ssp.* on *Fusarium moniliforme*.

Source	SD	Sum of squares	Mean of squares	F ratio	P value
Dose	2	74.07694	37.0385	11.1075	<0001*
Error	69	230.08292	3.3345		
Total	71	304.15986			

*P<0001 very important at the level

F. oxysporum f. sp. Considering the results of the effect of Dose-1 and Dose-2 on *Melongenae* colony development, it is significantly higher than the control dose. At the same time, the effect of Dose-1 and Dose-2 on colony development is almost the same. However, Dose-2 showed the most effective antifungal activity by a slight difference (Figure 2). Figure 3 shows the effects of different doses of applications on the colony development of different fungi.

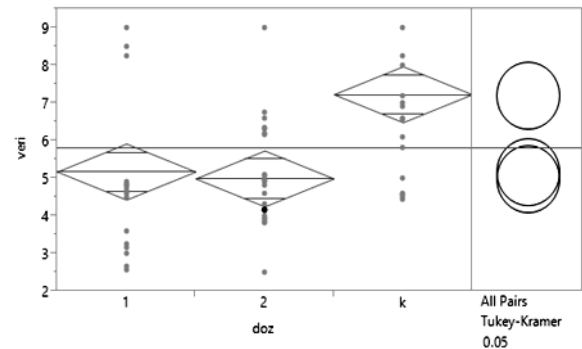


Figure 2. Different doses of *V. album ssp. album* extract against *F. oxysporum f. sp.* Comparison of the effect on *melongenae* colony development (applications, 1: 10mg/ml; 2: 20mg/ml; k: Control)

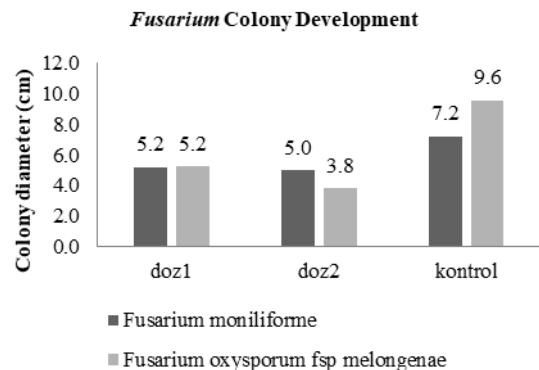


Figure 3. *Fusarium moniliforme* and *Fusarium oxysporum f.* developed two doses of *Viscum album ssp.* extract in Petri dishes. Sp. Effects of *melongenae* fungi on colony development (cm) (dose1: 10mg/ml, dose2: 20mg/ml)

Accordingly, different doses of *V. album ssp. album* extract are effective against both *F. melongenae* and *F. oxysporum f. sp.* Its effect on *melongenae* was found to be statistically significantly higher ($P < 0.001$) (Table 1 and Table 2).

Table 1 indicates that the effect of *V. album ssp. album* on *Fusarium moniliforme* produces quite different results. In other words, the *Fusarium moniliforme* fungus in the measured petri dishes shows different results from other measurements prepared at the same dose. This indicates that Dose-1 and Dose-2 used have antifungal activity.

Table 2. *Fusarium oxysporum f. of V. album ssp. album* Statistical results showing the effect on *melongenae*

Source	SD	Sum of squares	Mean of squares	F ratio	P value
Dose	2	114.38174	57.1909	24.6073	<0001*
Error	45	104.58639	2.3241		
Total	47	218.96813			

*P<0001 very important at the level

In Table 2, *Fusarium oxysporum f. of V. album ssp. album* was measured simultaneously. *sp. melongenae* at the same dose in petri dishes with *Fusarium oxysporum f. sp.* The development of the *Melongenae fungus* gives different results. For example, *Fusarium oxysporum f.*, grown in petri dish number 1 of Dose-1 measured at the same time. *sp.* While the diameter of the *Melongenae* fungus is 5.5/6.3, the diameter of the one in petri dish number 2 is 3.2/2.8. This

shows that Dose-1 and Dose-2 used have antifungal activity. The effects of low and high dose and control applications for each fungus were compared, and statistical changes in the effects of plant extracts at different doses were examined with Tukey's HSD test. Accordingly, no statistically significant difference was found between two doses of Mistletoe in *F. moniliforme* (Table 3).

Table 3. Statistical comparison of the effect of *V. album ssp. album* on the development of two different species at different doses.

Fungi	Applications		
	10mg/ml	20mg/ml	Kontrol
<i>Fusarium moniliforme</i>	5.175 b	4.992 b	7.229 a
<i>Fusarium oxysporum f.sp. melongenae</i>	5.228 b	3.792 c	7.628 a

*Means with different letters within the same row are statistically different according to Tukey's HSD test

On the other hand, *F. oxysporum f. sp.* In the *melongenae* fungus species, it is seen that both 10 mg and 20 mg doses inhibit the growth of this fungus at statistically different rates. When Table 3 is examined, it is understood that the herbal extract inhibited fungal growth in *Fusarium moniliforme* by 28.4% at low dose and 31% at high dose compared to the control. The same extract was used for *F. oxysporum f. sp.* The growth inhibitory effect on *melongenae* species was 31.5% at low and 50.3% at high doses. It was also examined whether there was a difference in effect between the two fungi and it was determined that *V. album ssp. album* extract affected both fungal species at similar rates (Table 4).

Table 4. Statistical comparison of the effect of *V. album ssp. album* on the development of two different fungal species

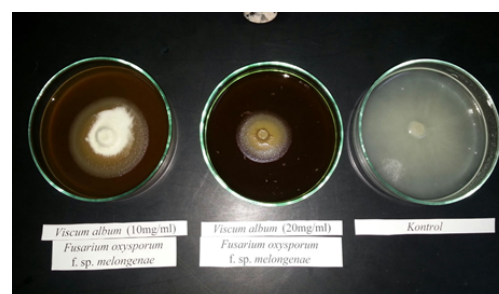
Fungus Type	Average Development (cm)
<i>Fusarium moniliforme</i>	5.799 a*
<i>Fusarium oxysporum f.sp. melongenae</i>	5.769 a*

*Means with the same letters in the same column are statistically insignificant according to Tukey's HSD test.

In Table 4, we see that *V. album ssp. album* is affected by *Fusarium moniliforme* and *Fusarium oxysporum f.sp. melongenae*; thus, Mistletoe had almost the same effect on both fungi. Figures 6 and 7 show the colony development of fungi in Petri dishes compared to the control.

**Figure 6.** Colony development of *Fusarium moniliforme*

When Figure 6 is examined, it can be seen that the width and length of the control dose completely covers the petri dish. (The width of a petri dish is 9 mm and the length is 9 mm). It is seen that Dose-1 prepared at 10mg/ml, spreads further compared to Dose-2, prepared at 20mg/ml. As can be seen from Figure 6, the antifungal property of Dose-2 is even greater than Dose-1.

**Figure 7.** *Fusarium oxysporum f.sp.* Colony development of *melongenae*

When Figure 7 is examined, it is seen that the Control dose covers the entire Petri dish. It is observed that Dose-1, prepared as 10mg/ml, spreads further compared to Dose-2, prepared as 20mg/ml. As can be seen from Figure 2, the antifungal property of Dose-2 is even greater than Dose-1.

When Figure 7 is examined, it is seen that the Control dose covers the entire Petri dish. It is observed that Dose-1, prepared as 10mg/ml, spreads further compared to Dose-2, prepared as 20mg/ml. As can be seen from Figure 2, the antifungal property of Dose-2 is even greater than Dose-1.

DISCUSSION

The findings of this study demonstrate that *Viscum album ssp. album* extracts possess significant antifungal activity against both *Fusarium moniliforme* and *Fusarium oxysporum f. sp. melongenae*. This aligns with existing literature highlighting the antimicrobial potential of plant-

based compounds, such as essential oils and extracts from other medicinal plants (Kordali et al., 2005; Peng et al., 2021). The dose-dependent inhibition observed in this study supports the hypothesis that increased concentrations of mistletoe extracts enhance antifungal efficacy. This pattern has also been observed in studies utilizing thyme and eucalyptus extracts, further corroborating the general applicability of this trend across plant species (Gilles et al., 2010; Şin et al., 2017).

The sensitivity of *Fusarium oxysporum f. sp. melongenae* to mistletoe extracts was notably higher than that of *Fusarium moniliforme*. This differential susceptibility could be attributed to variations in fungal physiology, cell wall composition, or metabolic pathways targeted by the bioactive compounds in *Viscum album*. Similar findings have been reported in studies examining the antifungal effects of *Artemisia* and *Cuminum* essential oils, where certain fungal species exhibited varying degrees of susceptibility (Kordali et al., 2005; Sakici et al., 2023).

While the current study focused on the antifungal properties of *Viscum album*, research on its mechanism of action remains limited. Bioactive compounds such as viscotoxin and lectins, which are known components of mistletoe, may interfere with fungal cell wall integrity or inhibit enzymatic processes critical for fungal growth (Szurpnicka et al., 2020). These hypotheses align with mechanisms proposed in studies of other plant-based antifungal agents and warrant further investigation.

The excessive use of synthetic fungicides has led to significant environmental and health concerns, including soil degradation, pesticide resistance, and contamination of water sources (Bricarello et al., 2023). As such, the development of eco-friendly alternatives like mistletoe extracts is a timely and necessary innovation. By demonstrating the efficacy of *Viscum album ssp. album* as a natural fungicide, this study contributes to a growing body of evidence supporting the use of plant-based solutions in integrated pest management systems (Parihar et al., 2022; Peng et al., 2021).

The practical application of mistletoe extracts in agriculture offers several advantages. First, the collection and preparation of extracts are cost-effective and do not require sophisticated equipment, making this approach accessible to farmers in low-resource settings. Second, the dual role of mistletoe as both a parasitic plant and a source of bioactive compounds offers a unique opportunity to repurpose a problematic species for beneficial use, thereby addressing ecological and agricultural challenges simultaneously.

While the results of this study are promising, several limitations should be addressed in future research. For instance, the study was conducted under controlled laboratory conditions, which may not fully replicate field environments where multiple biotic and abiotic factors interact. Additionally, the chemical composition of mistletoe extracts can vary depending on the host tree and environmental conditions, potentially affecting antifungal activity (Ayaz et al., 2023). Thus, further studies are needed to standardize extraction methods and evaluate the efficacy of mistletoe extracts across different agricultural contexts.

Future research should also explore the synergistic effects of mistletoe extracts with other plant-based antifungal agents. Such combinations could enhance efficacy while minimizing the required dosages, reducing the risk of phytotoxicity to crops. Moreover, studies investigating the potential impact of mistletoe extracts on non-target organisms, including beneficial soil microbiota and

pollinators, are essential for ensuring environmental sustainability.

This study addresses a significant knowledge gap in the antifungal potential of *Viscum album ssp. album*. By providing evidence of its efficacy against two economically important *Fusarium* species, this research contributes to the broader understanding of plant-based pest management strategies. It also highlights the potential for mistletoe, traditionally viewed as a problematic parasitic plant, to be repurposed as a valuable resource for sustainable agriculture.

CONCLUSIONS AND RECOMMENDATION

This study confirmed the antifungal efficacy of *Viscum album ssp. album* extracts, which demonstrated inhibitory effects exceeding 50% on the growth of *Fusarium oxysporum f. sp. melongenae*, particularly at higher doses. These findings highlight the potential application of mistletoe extracts as a natural, eco-friendly alternative to synthetic fungicides. The chemical components of *V. album ssp. album*, which inhibit fungal development, provide a promising basis for developing biopesticides aimed at protecting agricultural products from fungal diseases. However, it is essential to conduct further research to isolate and characterize these bioactive compounds, assess their synergistic effects, and better understand their specific modes of action against fungal pathogens.

The results also underline the importance of evaluating the broader ecological implications of using mistletoe extracts in agriculture. Before large-scale application, comprehensive studies are needed to examine the extracts' potential effects on crop health, soil quality, and non-target organisms, including beneficial microbes and pollinators. This step is crucial to ensure that the introduction of mistletoe-based treatments aligns with principles of sustainable and integrated pest management.

From a practical standpoint, this study demonstrates that mistletoe, typically regarded as a parasitic problem for farmers, can be transformed into a resource for agricultural benefit. By repurposing *V. album ssp. album* extracts for antifungal use, farmers could mitigate crop losses while reducing dependency on imported, expensive, and often harmful chemical pesticides. Moreover, the production of such natural fungicides domestically offers a significant opportunity for economic growth, providing cost-effective solutions for farmers and creating a new avenue for exports.

Moving forward, natural methods should take precedence over chemical approaches in combating plant pathogens. This approach not only addresses immediate agricultural challenges but also contributes to environmental conservation by reducing soil degradation and chemical runoff. Policymakers and agricultural researchers must prioritize the development of in vitro methods for producing affordable and effective biopesticides. By integrating these practices into national agricultural strategies, countries can foster self-reliance and reduce their reliance on imported agrochemicals. In conclusion, this study highlights the dual role of mistletoe as both a challenge and a solution in agriculture. The antifungal properties of *V. album ssp. album* extracts provide a foundation for their use as natural fungicides, aligning with global efforts to promote sustainable farming practices. Future research should focus on refining extraction methods, identifying specific bioactive compounds, and evaluating their long-term impacts on agricultural ecosystems.

Conflict of Interest

No conflict of interest was declared by the authors.

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