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EFFECTS OF ORGANIC AGRICULTURE PRODUCTS ON CORN SEED GERMINATION

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Abstract: This study evaluated the effects of two bird-repellent products, Repel Aves (methyl anthranilate, 30%) and Requiem Prime (terpenoid mixture), on corn seed germination and impact on radicle growth under laboratory conditions. Repel Aves showed that low concentrations (250–500 ppm) supported germination in the Harmonium hybrid, reaching nearly 100% by day 3, while higher concentrations (2000–6000 ppm) inhibited germination and radicle growth. For Turda 344 hybrid, high concentrations reduced germination to below 30%. Requiem Prime had a delayed effect on germination for Turda 344, with concentrations of 250–2000 ppm reaching 80-90% by day 4, while Harmonium showed accelerated germination at higher concentrations. These results suggest that bird-repellent products can offer seed protection while maintaining adequate germination, depending on concentration and hybrid.

Keywords: *germination, corn, terpenoids, methyl anthranilate.*

INTRODUCTION

Romania's temperate continental climate, along with the fertile soil found in many of its regions, has established agriculture as one of the country's primary economic activities. Corn remains the leading grain crop in the country in terms of both acreage and production. In recent years, Romanian farmers have increasingly focused on improving production efficiency and embracing smart agricultural practices. However, the lack of sufficient irrigation and hot, dry summers have led to considerable fluctuations in Romania's corn harvest. For instance, in 2022, the average yield was 3,297.7 kg/ha, with a harvested area of 2,437,220 ha, while in 2023, the yield increased to 3,981.6 kg/ha, though the area dropped to 2,196,110 ha (FAO, 2024).

One of the major challenges in agricultural production is protecting seeds from birds and pathogens. Biologically treated seeds are often exposed to bird consumption before germination, which compromises crop stability. Methyl anthranilate (30%) is a plant-derived food additive that acts as a taste repellent by irritating the trigeminal nerve, thereby preventing animals from consuming seeds. Its effectiveness in deterring crop consumption has been tested on various bird species (Blackwell et al., 2001; Avery et al., 1995; Avery & Decker, 1994; Mason et al., 1989; Mason & Clark, 1995). Limonene, a plant-derived monoterpene (Abrahim et al., 2000), has also been shown to function as a bird repellent (Clark, 1997). Terpenoid mixtures show significant potential as bird repellents due to their strong olfactory and gustatory properties, which are unpleasant for many bird species. These natural compounds, derived from plants, are known for their ability to act as defense agents against various pests, including insects and birds. Terpenoids such as limonene, citronellol, and eucalyptol emit intense aromas that discourage birds from approaching treated areas, thus reducing the risk of crop damage or contamination of industrial and urban spaces. The effectiveness of terpenoids as bird repellents can also be attributed to their sensory mechanisms. Birds have a more sensitive sense of smell than previously thought, and the volatile compounds can create olfactory discomfort, deterring them from staying in treated areas. Additionally, the bitter and unpleasant taste of certain terpenoids prevents the ingestion of treated seeds or other food sources, resulting in a dual repellent effect: through scent and flavour.

Two commercial products based on methyl anthranilate (30%) and a mixture of terpenoids (α-terpinene, p-cymene, d-limonene), were tested on corn seeds, and their impact on germination capacity was assessed to determine whether they could be used to efficient protection of crops without compromising the viability of the seeds.

MATERIAL AND METHODS

CORN HYBRIDS: Two corn hybrids were tested: Turda 344 (FAO 380) and Harmonium (FAO 380). The seeds were visually inspected, and those with visible cracks or damage were discarded. The selected seeds were placed in 50 ml conical tubes (25 seeds per tube), and the tubes were filled with 70% ethanol up to the 45 ml mark. The tubes were agitated by inversion for 3 minutes. After removing the ethanol, each tube was refilled with a 2% sodium hypochlorite solution and agitated again by inversion for 3 minutes. Once the sodium hypochlorite solution was removed, the seeds were rinsed five times with sterile deionized water, then left to dry at room temperature for 24 hours.

COMMERCIAL PRODUCTS: Two commercial products were tested: a bird repellent based on methyl anthranilate (30%) (Repel Aves, manufacturer Orange Saft), and a blend of terpenoids (α-terpinen: 59,7 %; p-cymen: 22,4 %; d-limonene: 17,9 %) (Requiem Prime, manufacturer Bayer) used for its repellent, insecticidal, and fungicidal effects.

The products were applied at doses of 250, 500, 1000, 2000, 3000, 5000, and 6000 ppm (µl active substance per kg of seed). The treatment solution was prepared according to the doses, mixed until homogeneous, and then the seeds were added (20 seeds per repetition, with 5 repetitions). The seeds were left in contact with the solution for 30-45 minutes, then drained and placed to dry on perforated trays for 48 hours at room temperature (25°C) (Figure 1). The control consisted of seeds treated with sterile distilled water.

Figure 1. Corn seeds are dried on perforated trays after treatment with the two commercial products

The treated and dried seeds were placed in Petri plates (9 cm in diameter), lined at the bottom with sterile filter paper previously moistened with 3 ml of sterile water. The plates were incubated at 25°C for 4 days. To maintain humidity, 3 ml of sterile distilled water was added to each Petri dish every 3 days. Observations on the number of germinated seeds were made daily, and after 7 days, the radicle length was measured (Figure 2 and Figure 3). Seeds were considered germinated when the radicle and plumule reached approximately 0.2 cm in length.

The statistical analysis of the data was performed using GraphPad Prism software version 7.05. To compare differences between groups, Welch's t-test was applied, which is recommended for cases where group variances are unequal.

Figure 2. Germinated corn seeds

Figure 3. Measuring radicle length using an electronic caliper

RESULTS

Impact of the treatment with the commercial product Repel Aves based on methyl anthranilate (30%) on corn seed germination.

The germination of corn seeds from the Turda 344 hybrid began to be observed on the third day after the seeds had been placed in the Petri dishes, but it was not completed within the observation period. The control showed a steady increase in germination, reaching approximately 80% on the fifth day. V1 (the first concentration) displayed a similar pattern to the control, with a rising germination rate and about 75% germination on the fifth day (Fig. 4). The V2 to V4 variants showed moderate germination progress but with lower performance than the control and V1, reaching 40-60% by the last day of observations.

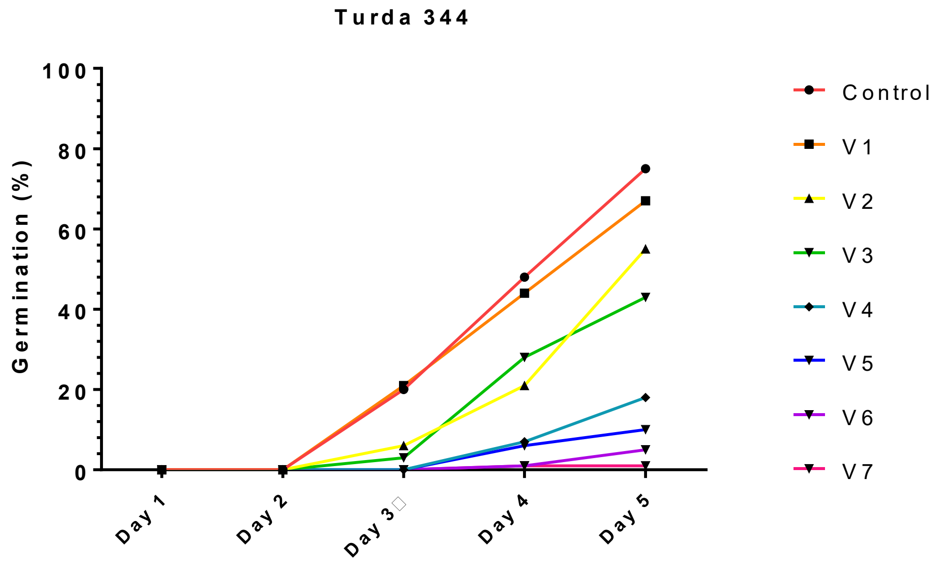

Figure. 4. Germination dynamics of Turda 344 corn hybrid treated with different concentrations of methyl anthranilate-based product

The control showed rapid germination for the Harmonium hybrid, reaching almost 100% by the second day. V1 to V4 exhibited similar performance to the control, with fast and high germination close to 100% in the first few days. V5 to V7 showed delayed germination, with much slower growth, especially V6 and V7, which had the lowest germination rates by day five. The impact of the methyl anthranilate-based commercial product on corn seed germination appears to be concentration-dependent. Lower concentrations (V1 and V2) had a positive or neutral effect on germination, especially for the Harmonium hybrid, while higher concentrations (V5-V7) exhibited an inhibitory effect (Fig. 5).

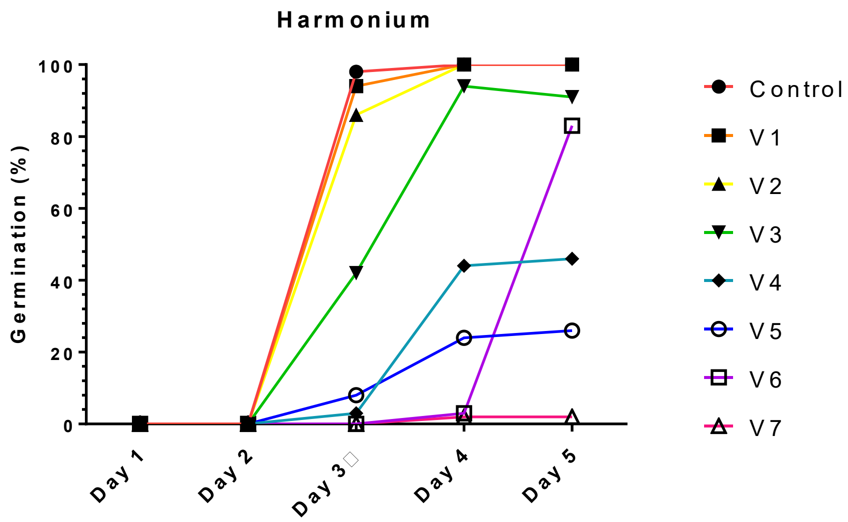

Figure. 5. Germination dynamics of Harmonium corn hybrid treated with different concentrations of methyl anthranilate-based product

Impact of the treatment with the commercial product Requiem Prime, based on a terpenoid mixture (α-terpinen: 59,7 %; p-cymen: 22,4 %; d-limonene: 17,9 %) *on corn seed germination.*

In the first two days, the germination rate of Turda 344 hybrid seeds was very low or non-existent for all treatments (control and V1-V7) (Figure 6). Starting on day three, a sharp and strong increase in germination was observed, particularly for V2, V3, V4, and V5, all reaching nearly 80-100% by the end of the observation period. Concentrations V1 and V6 showed lower germination compared to the other treatments. Although all treatments reached similar germination levels by the end of the observation period, germination for V1 and V6 was slightly delayed. Untreated seeds had a germination rate similar to the treated variants, suggesting that terpenoid treatments may not offer a significant advantage for this hybrid, or the effect is minimal.

For the Harmonium hybrid, germination occurred rapidly and almost simultaneously for all treatments, especially on the second and third days (Figure 7).

All variants, including the control, reached nearly 100% germination by day three, indicating that terpenoid concentrations did not have a distinct effect on germination for this hybrid. The germination rate was very high across all treatments. The results suggest that the terpenoid mixture does not strongly influence corn seed germination for the Harmonium hybrid, but depending on the concentration, it may cause a slight delay for Turda 344.

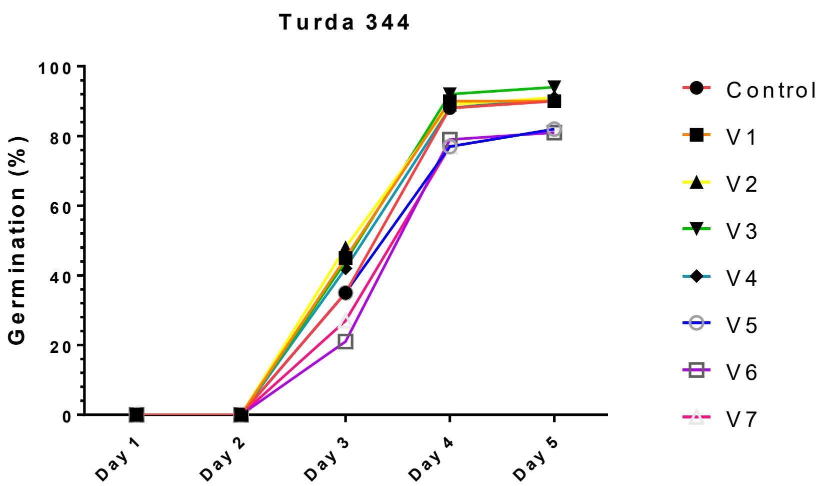

Figure 6. Germination dynamics of Turda 344 corn hybrid treated with different concentrations of the terpenoid-based product

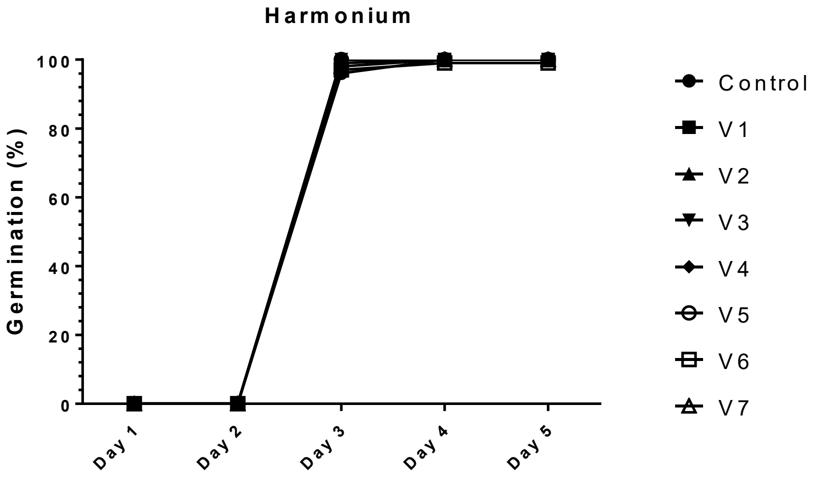

Figure 7. Germination dynamics of Harmonium corn hybrids treated with different concentrations of the terpenoid-based product

Results on the impact of treatment with the commercial product Repel Aves based on methyl anthranilate (30%) on the radicle length of corn seeds.

For the Turda 344 hybrid, the control showed radicle lengths of approximately 2.5 cm (Figure 8).

As the methyl anthranilate concentration increased (V1-V7), a drastic decrease in radicle length was observed. Treatments with lower concentrations (V1-V4) reduced radicle length but still allowed moderate growth, whereas higher concentrations (V5-V7) reduced radicle length to nearly zero. This suggests that Turda 344 hybrid is sensitive to high concentrations of methyl anthranilate, with root growth negatively affected as the dose increases.

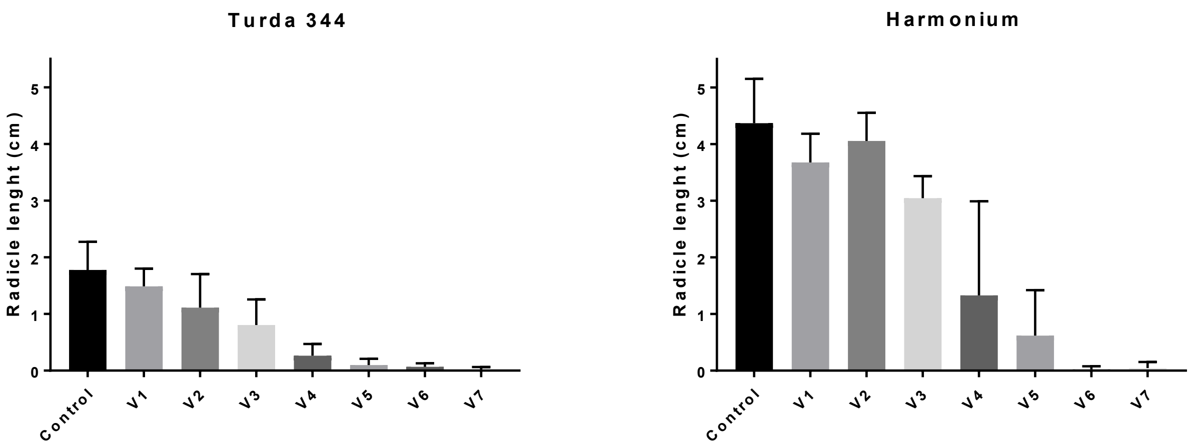

Figure 8. The radicle length of corn seeds from both hybrids treated with different concentrations of the methyl anthranilate-based product

For the Harmonium hybrid, the control showed longer radicles, around 4 cm, indicating better initial growth compared to Turda 344. In treatments with the first three concentrations (V1-V3), radicle length decreased, but not as drastically as in Turda 344, suggesting a greater tolerance to lower concentrations of methyl anthranilate. Similar to Turda 344, treatments with higher concentrations (V5-V7) completely inhibited root growth, showing that at high concentrations, methyl anthranilate has a similarly negative effect on this hybrid.

In conclusion, low concentrations of methyl anthranilate (V1-V3) moderately affected radicle growth, especially in the Harmonium hybrid, which showed slightly better tolerance compared to Turda 344. High concentrations (V5-V7) had a strong inhibitory effect on root growth in both hybrids, suggesting increased toxicity of the commercial product at high doses. These results highlight the importance of selecting an appropriate methyl anthranilate concentration to avoid compromising root development, particularly in sensitive plants like the Turda 344 hybrid.

Results on the impact of treatment with Requiem Prime, a terpenoid blend-based product, on corn seedling radicle length.

For the Turda 344 hybrid, the control (untreated) recorded an average radicle length of approximately 4 cm (Figure 9). Treatments V1-V7 showed similar radicle lengths compared to the control, with slight variation. All treatment values (V1-V7) were relatively close to each other, with radicle lengths ranging between approximately 3 and 4 cm. No significant decrease in radicle length was observed between the control and treatments, suggesting that the terpenoid blend had little impact on radicle growth for this hybrid within the tested concentration range.

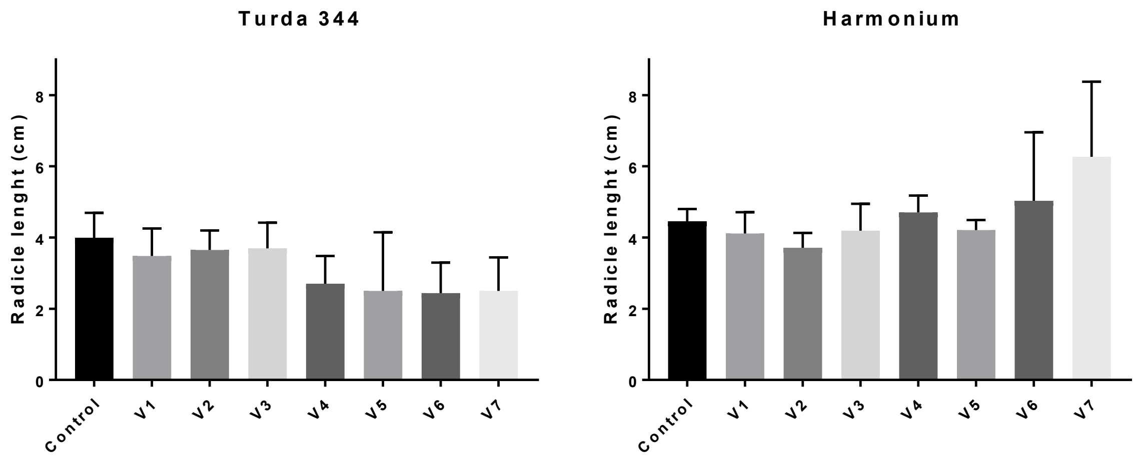

Figure 9. Radicle length of corn seeds from the two hybrids treated with different concentrations of a terpenoid blend-based product

For the Harmonium hybrid, the control showed a radicle length of around 4 cm. The first treatments (V1-V4) had a minimal effect on radicle length, with values remaining close to those of the control, ranging from 3 to 4 cm, indicating a low impact of terpenoids on radicle growth at these concentrations. However, with treatments V5, V6, and V7, the radicle length peaked at the highest tested concentration, reaching approximately 7 cm. Compared to the control, treatments V6 and V7 were statistically significantly different (t=0.66 and t=1.89, respectively), with root growth improving by around 13% and 40.9%. This trend suggests a stimulating effect of higher terpenoid concentrations on radicle growth. Such substantial growth may indicate better initial plant development, potentially leading to enhanced nutrient and water uptake, as well as increased resilience to environmental stress. Overall, these results are promising and suggest that treatment with this terpenoid-based product may benefit corn seedling germination and early development. However, definitive conclusions would require further studies involving multiple replicates, statistical analysis, and long-term assessments of its impact on yield and overall crop health.

In conclusion, higher doses of terpenoids appear to stimulate radicle growth, while lower doses have a reduced impact.

The observed differences in the responses of the two corn hybrids to treatments with terpenes and methyl anthranilate can be attributed to a combination of genetic, physiological, and biochemical factors. The Harmonium hybrid exhibited greater resistance to external influences, maintaining a constant germination rate regardless of concentration, while Turda 344 was more sensitive to higher concentrations of the compounds, leading to slower or inhibited germination. From a genetic perspective, each hybrid has a different profile, influencing their response to external treatments. Harmonium can be considered more genetically robust, able to maintain rapid and uniform germination despite varying concentrations. In contrast, Turda 344 appears more sensitive to stress induced by terpenes and methyl anthranilate, especially at higher concentrations (V5-V7), which may be linked to a greater genetic susceptibility to these substances.

Another factor that may influence the different responses of the two hybrids is the absorption rate of the treatment. Turda 344 may absorb terpenes and methyl anthranilate more quickly or in larger quantities, leading to toxic or inhibitory effects at higher concentrations. On the other hand, Harmonium might benefit from more efficient absorption regulation mechanisms, preventing interference with the germination process. Additionally, tolerance to oxidative stress plays an important role. Harmonium appears to have a greater capacity to manage oxidative stress due to more effective antioxidant mechanisms, while Turda 344 may be more vulnerable to this type of stress, thus explaining the inhibition of germination at higher concentrations of terpenes and methyl anthranilate.

Differences in hormonal responses could also contribute to these observations. The treatment with terpenes and methyl anthranilate may influence the balance of plant hormones, such as abscisic acid (ABA) and gibberellins (GA), which regulate the germination process. Harmonium may have more efficient hormonal control, maintaining rapid germination regardless of treatment, while Turda 344 may exhibit greater sensitivity to hormonal imbalances, slowing germination at higher concentrations. Furthermore, the seed structure may explain the different responses of the two hybrids. Harmonium seeds may have a more robust seed coat structure, acting as a protective barrier against the treatment and maintaining consistent germination. In contrast, Turda 344 seeds may have greater permeability, allowing the treatment to penetrate more quickly and exposing them to higher concentrations of active compounds. Although the experiments were conducted under similar conditions, it is important to note that small variations in environmental conditions, such as humidity and temperature, may have had a different impact on the two hybrids, affecting germination and their interaction with the applied treatments.

Methyl anthranilate has shown considerable potential as a repellent for both western corn rootworm larvae and birds, suggesting its dual role in pest management. Bernklau et al. (2016) identified methyl anthranilate as an active compound in maize root extracts that effectively repelled western corn rootworm larvae. At concentrations of 1, 10, and 100 μg, methyl anthranilate significantly deterred larvae from approaching attractive carbon dioxide sources, indicating its potential use in a push-pull control strategy for managing these pests.
Although not directly tested on corn seeds, methyl anthranilate has demonstrated efficacy as a bird repellent, with concentrations ≥1.0% shown to reduce bird damage in agricultural contexts (Mason et al., 1991). Its repellent properties have been observed against species like red-winged blackbirds and European starlings, although habituation was noted in red-winged blackbirds

after prolonged exposure (Mason et al., 1991). This is in contrast to anthraquinone, another repellent, which has been more extensively studied and used as a seed treatment to mitigate bird damage in crops, including corn (Deliberto & Werner, 2016).

Field trials have confirmed methyl anthranilate's effectiveness in reducing bird damage to corn fields, with treated fields exhibiting significantly less damage compared to untreated ones (Askham, 2000). Furthermore, controlled environment studies revealed that methyl anthranilate could deter birds such as house sparrows from consuming treated maize seeds and seedlings (Esther et al., 2011; Ahmad et al., 2018). These results highlight the compound's broad potential in agricultural pest control. Commercial formulations of methyl anthranilate, such as ReJeX-iT and Bird Shield, are already in use for crop protection (DeLiberto & Werner, 2024; Moran, 2001; Müller-Schwarze, 2009).

However, several considerations and limitations should be addressed. The efficacy of methyl anthranilate varies between bird species, with some birds potentially habituating to the compound, reducing its long-term effectiveness (Mason et al., 1991; Moran, 2001). Moreover, while promising in controlled conditions, the compound's effectiveness in natural field settings can be inconsistent, necessitating further research to optimize its application (Esther et al., 2011; Ahmad et al., 2018).

In terms of its effects on corn seed germination, methyl anthranilate was found to slightly reduce germination rates. Treated seeds had a germination rate of 90%, compared to 96% for untreated seeds, suggesting a minor but notable impact on germination (Lacy et al., 2018). This indicates that while methyl anthranilate can be an effective repellent, its potential negative effects on seed germination should be considered in its application.

Overall, methyl anthranilate offers a viable approach for repelling birds from corn seeds and other crops, with demonstrated effectiveness in reducing bird damage. However, its success in field conditions and across different species remains variable, and additional research is necessary to further refine its use and maximize its benefits in agricultural pest management.

CONCLUSIONS

The Harmonium hybrid demonstrated greater resistance to treatments with terpenoids and methyl anthranilate, showing rapid germination and significant root growth. In contrast, Turda 344 was more vulnerable to higher concentrations, suggesting that hybrid selection can influence the success of these treatments. High concentrations of methyl anthranilate had a notably toxic effect on germination and growth, particularly for Turda 344. For terpenoids, moderate concentrations were beneficial, while high concentrations inhibited growth, except for the Harmonium hybrid, where the highest concentration stimulated growth. These findings highlight the importance of determining an optimal dose to maximize benefits while minimizing negative effects.

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