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THE IMPACT OF GLYPHOSATE ISOPROPYLAMINE SALT AND GLUFOSINATE-AMMONIUM ON SOME SOIL PATHOGENS CAUSING DISEASE IN WHEAT

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Abstract

Glyphosate isopropylamine salt and glufosinate-ammonium are commonly used herbicides for presowing or host-harvest weed control in Turkish wheat fields. Although many studies have been conducted on their efficacy against weeds, limited studies are available in the literature on their impact on soil pathogens causing disease in wheat, such as *Fusarium culmorum, Gaeumannomyces graminis* var. *tritici,* and *Bipolaris sorokiniana*. In this in vitro study, the efficacy of glyphosate isopropylamine salt and glufosinate-ammonium on the mycelial growth of these pathogens grown in PDA was investigated. Changes in the mycelial growth of fungal species grown in herbicide-amended PDA medium and control medium were observed by measuring the radial growth of the colonies three times per week. Glufosinate ammonium effectively suppressed the fungal growth of all of the pathogens tested in vitro. Glyphosate isopropylamine salt induced mycelial growth of *B. sorokiniana*; however, there was little or no impact of the herbicide on other pathogens tested. Wheat growers should be careful using glyphosate isopropylamine salt in fields that are infected by *B. sorokiniana,* or they should use glufosinate-ammonium in fields where these pathogens are common.

Key words: herbicide, glyphosate, glufosinate, soil-borne pathogen, in vitro

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Introduction. Herbicides have been considered an indispensable component in modern agricultural systems in recent decades because they can control weeds that cause yield and quality losses in agricultural crops. There are some problems related to the agro ecological environment based on herbicides, such as resistance, carryover, drift, leaching, decreasing biodiversity, and chance microbial communities in the soil. Herbicide-resistant biotypes are the most significant issue in agricultural fields because they cannot commonly be controlled by herbicides, and they steadily replace sensitive biotypes year by year [1]. Carryover of herbicide can be a troublesome case if herbicide rates are applied over their recommended doses or some factors may delay their degradation process, including drought, soil type, and organic matter content of the soil-applied [2]. Similar to carryover, leaching has heavily occurred under some extreme environmental conditions, such as heavy rainfall [3]. Although it can be an easily controllable factor among these problems, drift may cause crop injury if adverse environmental conditions occur [4]. Decreasing biodiversity in agricultural fields is actually the sum of the side impacts of agricultural treatments, especially herbicides [5]. The impact of herbicides on the soil microbial community may be the least studied subject. The problems related to herbicide use are increasing daily, especially for total herbicides.

Total herbicides can effectively control many weed species in orchards and fallow, agricultural and nonagricultural fields. Glyphosate isopropylamine salt and glufosinate are the most common broad-spectrum herbicides in many countries, especially in fields sown with GMO crops [6]. They can be used in conventional agricultural fields pre-sowing and postharvest to clean the fields [7] or post-emergent using hooded sprayers due to their low selectivity potency [8]. These herbicides have also been applied to control weeds in reduced-tillage or no-tillage farming systems that are heavily reliant on them. The fields where these herbicides were used were commonly infested by some soil fungal pathogens.

Soil-borne fungal pathogens are among the most important diseases caused by take-off or severe yield loss in Turkish wheat fields [9]. Herbicide negatively or positively affects the soil microbial community depending on the species. Mekwatanakarn and Sivasithamparam [10] indicated that the saprophytic growth of *Gaeumannomyces graminis* var. *tritici* in soil was slightly decreased by glyphosate isopropylamine salt, but this impact was not statistically significant. Zobiole et al. [11] reported that an increase in the glyphosate isopropylamine salt rate increased *Fusarium* spp. colonization on the root because of the suppressive impact of glyphosate isopropylamine salt on the plant defense system. In contrast to glyphosate isopropylamine salt, glufosinate can strongly suppress most fungal organisms. Albrecht and Kortekamp [12] found that glufosinate ammonium resulted in a reduction in mycelial growth or spore production of some pathogens, including *Botrytis cinerea, Guignardia bidwellii, Penicillium expansum* and *Phomopsis viticola.*

Glyphosate isopropylamine salt and glufosinate-ammonium are commonly used herbicides for pre-sowing or host-harvest weed control in Turkish wheat fields. The aim of this study was to determine the impact of these herbicides on the mycelial growth of economically important soil pathogens of Turkish wheat fields, such as *Fusarium culmorum, Bipolaris sorokiniana,* and *Gaeumannomyces graminis* var. *tritici* in vitro experiment.

Materials and methods. Fungal species (*F. culmorum, B. sorokiniana,* and *Gaeumannomyces graminis* var. *tritici*) were isolated from wheat plants that showed disease symptoms. These fungal pathogens were placed on potato dextrose agar (PDA) and incubated for 7 days at 24 °C ± 1 °C to isolate single spores. Spore suspensions were prepared using isolates grown on PDA and transferred to water agar (WA – 2%) for 24 h at 24 °C ± 1 °C. Spore disks taken from WA using a sterile cork borer were placed on PDA following incubation at 24 °C ± 1 °C for 4–7 days. Fungal species were identified based on their macroscopic features, such as conidial shape, colour, texture, topography, or hyphal structures.

A herbicide-PDA mixture was used as a growth medium to determine the impact of herbicides on the mycelial growth of fungi. PDA was prepared and sterilized in an autoclave (121 °C, 20 min) and placed into a sterile cabinet to cool to 45—50 °C. Glyphosate isopropylamine salt and glufosinate-ammonium rates were added to PDA and mixed thoroughly on a hotplate, followed by pouring into Petri dishes. Herbicides were adjusted to X/2, X, and 2X (X: recommended rates of herbicides, which equal 7.2 and 3 mg ai mL^{-1} for glyphosate isopropylamine salt and glufosinate-ammonium, respectively). Petri dishes were sealed with parafilm and incubated at 24 °C ± 1 °C until they were covered by mycelial growth. Each treatment was replicated four times.

Changes in the mycelial growth of fungal species grown in herbicide-supplemented PDA medium and control medium were observed by measuring the radial growth of fungal colonies one time in 2–3 days. Colony diameters of the fungal pathogens were measured using an electronic digital caliper. The time required to cover the Petri dish with fungal pathogens was calculated and used for comparisons. The data from in vitro studies were analyzed by ANOVA. The means were separated by Duncan's Multiple Range Test using SPSS statistical program Ver 23.0.

Results and discussion. Mycelial growth of *F. culmorum* was not affected by the presence of glyphosate isopropylamine salt. Colony expansion of the pathogen in herbicide-supplemented medium was nearly the same as that in the control, and the pathogen fully covered the Petri dishes on the eighth day (Fig. 1). Glufosinate ammonium significantly suppressed the fungal growth of *F. culmorum* compared to the control, but the impact of herbicide rates on growth was similar to each other and statistically insignificant (Fig. 4a). Fernandez et al. [13] indicated that glyphosate might increase the severity of *Fusarium* cereal diseases under field conditions because of the increasing fungal colonization of

Fusarium or suppression of the plant defense system and other fungi associated with pathogenicity or commensalism. Contrary to their results, no suppressive or inductive impact of glyphosate on the mycelial growth of *F. culmorum* was observed in our experiment due to a lack of competition. Indeed, these differences between the studies are not surprising because Fernandez et al. [13] indicated that the suppressive impact of glyphosate on other fungi and plant defense systems may increase the severity of *Fusarium* infections in general. Carranza et al. [14] reported that glyphosate increased in growth and disease severity of *Fusarium* sp. strains isolated from maize specifically, such as *F. oxysporum* and *F. verticillioides* in vitro conditions. These two studies' findings were similar to our results.

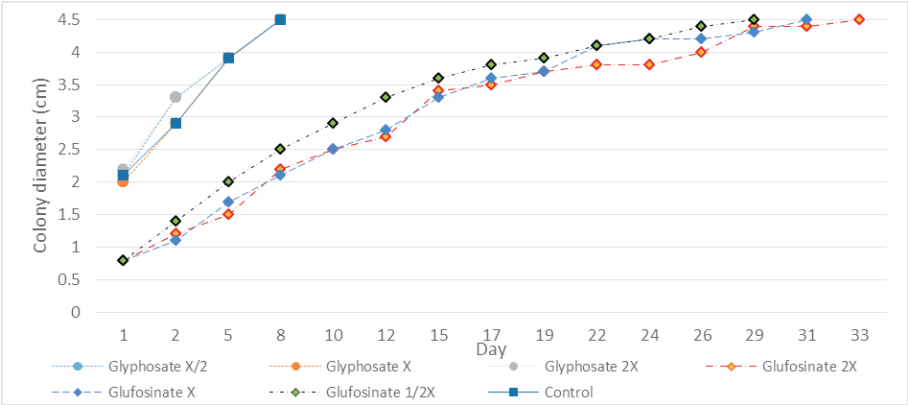

Fig. 1. Impact of glyphosate isopropylamine salt and glufosinate-ammonium rates on mycelial growth of *F. culmorum*

Glufosinate presence in vitro distinctly retarded colony growth regardless of herbicide dose (Fig. 1). The herbicide reduced the colony growth of *F. culmorum* by 73–76%. No available literature was found about the efficacy of glufosinate ammonium on the growth of *F. culmorum*. Therefore, this finding will be the first report associated with the glufosinate-ammonium – *F. culmorum* relationship.

Gaeumannomyces graminis var. *tritici* was positively affected by glyphosate presence in the growing media. Its growth increased as the glyphosate rate increased, but this inductive impact did not significantly change the time required to cover the Petri dishes by *Gaeumannomyces graminis* var. *tritici* (Fig. 2). Mekwatanakarn and Sivasithamparam [10] indicated that saprophytic growth of *Gaeumannomyces graminis* var. *tritici* in soil was slightly decreased by glyphosate isopropylamine salt even though the impact was not statistically significant. Baley et al. [15] also reported similar results to Mekwatanakarn and Sivasithamparam [10] and our findings. In the second herbicide, glufosinate-ammonium presence resulted in a decline in mycelial growth with an increase in herbicide rates (Fig. 4b). Launching of mycelial growth in glufosinate-ammonium-amended PDA was

delayed depending on the increasing herbicide rate. The increase in glufosinate dose resulted in reduced fungal growth at 18–44% ($P < 0.05$). Although little research has been conducted on the impact of glyphosate isopropylamine salt, to date, no data have been presented with respect to the relationship between glufosinate ammonium and *Gaeumannomyces graminis* var. *tritici*.

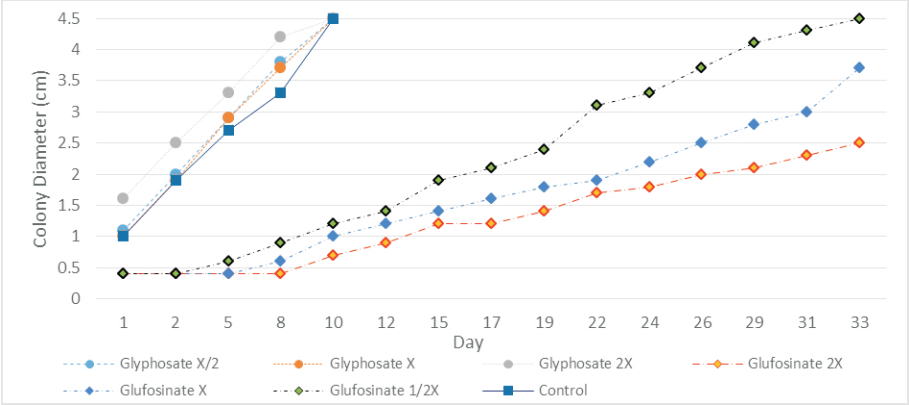

Fig. 2. Impact of glyphosate isopropylamine salt and glufosinate-ammonium rates on mycelial growth of *Gaeumannomyces graminis* var. *tritici*

The response of *B. sorokiniana* to glyphosate isopropylamine salt was more apparent than that of *Gaeumannomyces graminis* var. *tritici* and *F. culmorum*. The increase in glyphosate dose resulted in increasing fungal growth and a decrease in the time required to cover the Petri dishes by the fungi (Fig. 3). In contrast to glyphosate isopropylamine salt, glufosinate suppressed fungal growth in vitro. The suppressive impact increased in severity with the increase in glufosinate dose and reached 60% suppression when applied at a 2X rate (Fig. 4c). Although a few pa-

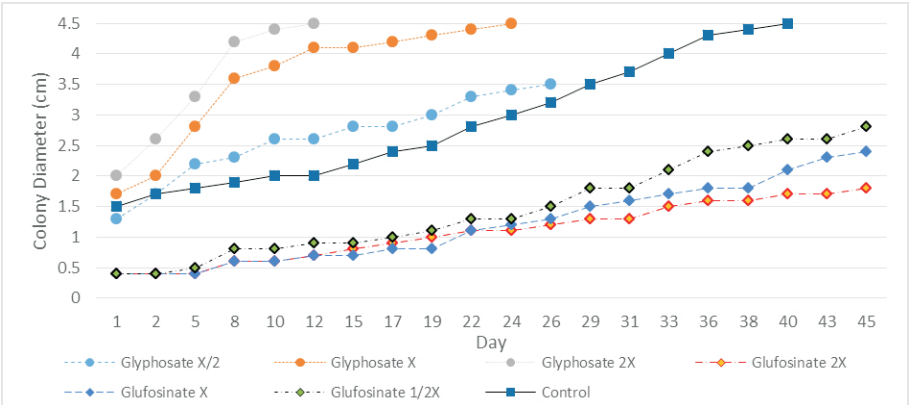

Fig. 3. Impact of glyphosate isopropylamine salt and glufosinate-ammonium rates on mycelial growth of *B. sorokiniana*

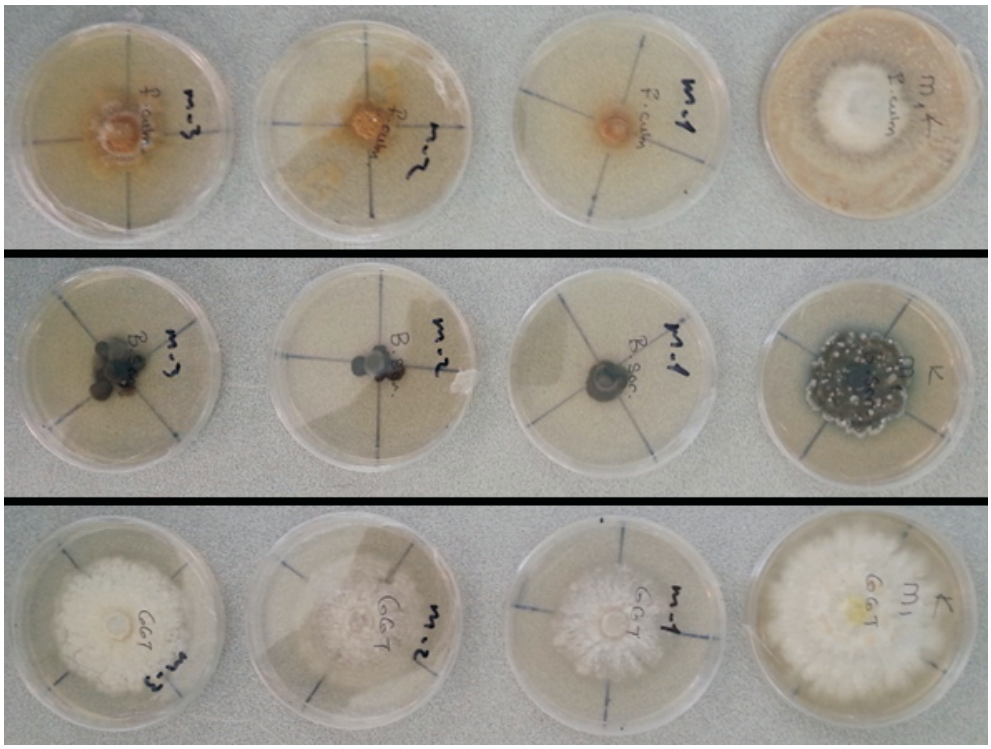

Fig. 4. Impact of glufosinate-ammonium rates on mycelial growth of the fungal pathogens (K: Control; M-1: 6 mg ai mL^{-1}; M-2: 3 mg ai mL^{-1}; M-3: 1.5 mg ai mL^{-1}; Top: *B. sorokiniana*; Middle: *Gaeumannomyces graminis* var. *tritici*; Bottom: *F. culmorum*)

pers have documented the efficacy of some herbicides on this fungal pathogen, no study was found in the literature about the impact of glyphosate isopropylamine salt and glufosinate-ammonium on it [16].

Briefly, herbicide users have mainly focused on the suppressive or lethal impacts of herbicides on weeds. However, new findings about the side effects of these agrochemicals on nontarget organisms indicated that herbicides may change the environment involved. However, our knowledge about these matters is still very limited. The impact of glufosinate ammonium on the mycelial growth of *F. culmorum, Gaeumannomyces graminis* var. *tritici,* and *B. sorokiniana* showed that this herbicide retarded the growth of these soil pathogens under in vitro conditions. In contrast to glufosinate ammonium, glyphosate isopropylamine salt stimulated the mycelial growth of *B. sorokiniana*, but no significant change in mycelial growth was observed in other pathogens. New studies should be conducted under field conditions to determine how environmental conditions affect the susceptibility of soil fungal pathogens to these herbicides. Wheat growers should be careful using glyphosate isopropylamine salt in fields that are infected by *B. sorokiniana,* or they should use glufosinate-ammonium in fields that are common.

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