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Characterizing the Utility of Atonik on Improving Rice Response to Herbicides

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Crop, Soil and Environmental Science

by

Srikanth Kumar Karaikal University of Agricultural Sciences Bachelor of Science in Agriculture, 2018

May 2023 University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

Nilda Roma Burgos, Ph.D. Thesis Director

Trenton Roberts, Ph.D. Committee Member Vibha Srivastava, Ph.D. Committee Member

Andy Mauromoustakos, Ph.D. Committee Member

Abstract

Biostimulants can improve plant tolerance to abiotic stress by improving plant growth and development. Herbicides cause abiotic stress to crops shortly after application, which may affect yield. Preliminary tests showed that rice yield benefits from Atonik seed treatment or tank-mixture of Atonik with foliar herbicides. Experiments were conducted in the field and greenhouse to: (1) determine if seed treatment with Atonik improves rice tolerance to pre-plant herbicides; (2 and 3) evaluate the effect of Atonik seed treatment on rice response to pre-emergence and delayed pre-emergence herbicides; and (4) determine if Atonik improves crop safety of foliar-applied herbicides. Field studies were conducted in silt loam and clay loam soil in Rohwer, AR except the foliar tank-mix study (silt loam only). Greenhouse studies were conducted in Fayetteville, AR. The greenhouse experiments were conducted using silt loam soil from Fayetteville, AR, and the experimental design was a split-plot randomized complete block design with four replications. Greenhouse study 1 had seven levels of Atonik ranging from 0 to 3.5 ml kg⁻¹ seed with increments of 0.5 ml kg⁻¹ of seed. The herbicides tested were the same as in the field. The field study (1) was a seed treatment x preplant herbicide test with 24 treatments, with herbicide (12 levels) as whole plot and Atonik concentration (2 levels) as subplot factors. Atonik rates were 0 and 0.225% v/wt of seed. The experimental design was split-plot randomized complete block with four replications. In both soil types, injury was less than 10% except with Canopy and Trivence, which caused > 60% injury. Seed treatment with Atonik did not reduce injury, and generally did not benefit rice yield in clay loam soil; however, in the silt loam soil yield increased numerically from 1043 to 1882 kg ha⁻¹ in 9 of 12 treatments (mean = 1294 kg ha⁻¹). The field study (2) was a seed treatment x pre-emergence herbicide test with 20 treatments, with herbicide (4 levels) as whole plot and Atonik concentration (5 levels) as subplot factors. Atonik rates were: 0, 0.375, 0.75, 1.5, and 3.0 ml kg⁻¹ seed 'RT7321FP'. In both soil types, injury was generally <40% for all treatments except for quinclorac + pendimethalin which caused > 60% injury. Seed treatment with Atonik did not reduce injury and generally did not benefit rice yield. The yield was numerically highest (12,500 kg ha⁻¹) with the nontreated check. In the greenhouse (1) pre-emergence test, the highest biomass (9g 2plants⁻¹) was produced with 2.5 ml kg⁻¹ seed treatment without herbicides. However, in the delayed pre-emergence study,

the highest biomass was obtained with quinclorac + thiobencarb applied to rice treated with 1.0 ml kg⁻¹ seed. A field study (4) was conducted with foliar herbicides (11 levels) as wholeplot and Atonik concentration (0, 0.075 and 0.225 % v/v) as subplot factors. Atonik was tank-mixed with herbicides and applied to V3 rice 'RT7321FP'. Overall, the application of Atonik with foliar rice herbicides did not increase yield significantly regardless of Atonik rate. Plots treated with quinclorac + propanil without Atonik produced the highest yield (13491 kg ha⁻¹). This yield was 18% higher than the nontreated check. In the greenhouse study (2), the highest biomass (30g 3plants⁻¹) was produced when rice plants were treated with 0.075% v/v Atonik without herbicide. Overall, these experiments indicated that the benefit from Atonik is small or none and is herbicide dependent.

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Chapter 1

General Introduction

Rice overview

Rice descended from a wild grass at least 130 million years ago and was domesticated in Asia around 9,000 years ago (Kush, G.S, 1997). Rice (*Oryza sativa* L.) belongs to the *genus Oryza* and the family Poaceae (Kellogg, 2001). Rice is divided into three major subgroups: *indica*, *japonica*, and *javanica*. Among these subgroups, *indica* is widely grown in Asia (Khush,1997). Rice is a crop of tropical climate; however, it is also grown successfully in humid and sub-humid regions under subtropical and temperate climate during the summer season. Rice is a semi-aquatic plant, primarily grown in flooded culture. In some areas rice is grown as a rainfed crop but yields under these conditions are significantly lower than in flooded culture. About half of the world's population consumes rice as a staple in their diet, which accounts for 20% of all calories consumed worldwide (Kubo and Purevdorj 2004). The cultivation of rice in the United States began in 1685, via what was then the maritime trade route from Madagascar into the Charles Towne Harbor, South Carolina, US. In modern times, the countries with the highest rice production are China (149 MT), India (130 MT), Bangladesh (36 MT), Indonesia (34.6 MT) and Vietnam (27.4 MT) (USDA-FAS 2022).

Importance of rice in the US

In 2021, rice was planted on 934,000 ha (2,307,964 acres) producing 5,541,000 MT (million tons) of rough rice (USDA-NASS 2022). The US rice growers produce almost 9,071,847 MT of rice in Arkansas, Louisiana, California, Mississippi, and Texas. The US consumes about half the rice it produces; the other half is exported, mostly to Mexico, Central America, Northeast

Asia, and the Middle East. Globally, US rice trading occupies the 11th position in the world and the rice contribution of \$1.92 billion to the US economy (USDA-FAS 2022).

Rice Production in Arkansas

Rice production in Arkansas began in 1902 in 0.404 ha in Lonoke County, and it is now grown in 40 of the state's 75 counties, primarily in the eastern half (Hardke, 2020). In 2021, Arkansas rice growers harvested 1,194,000 acres, accounting for 47.5% of total US rice production with an average of 8552.0 kg ha⁻¹ of rice grain (166.7 bushels/acre; USDA-NASS-2021). Most rice in Arkansas (53%) is planted under conventional tillage (Hardke 2020). The seedbed preparation involves fall tillage when the weather allows, followed by spring tillage. Rice is produced in three soil types: silt loam (50.7%), clay (25.5%), and clay loam (20.8%). Rice is mostly grown in rotation with soybean (*Glycine max* (L). Merr), which accounts for 67.7% of all rice hectares (Hardke 2020). The rice planting period ranges from the last week of March to early June, but the optimum period is between April and mid-May. About 85% of the rice planted is drill-seeded (~5%). In Arkansas, rice cultivars include long-grain (about 90%), medium-grain, and short-grain varieties (USDA-NASS, 2021).

Rice weed control

A major limitation to rice production is weed control. The world's most problematic weeds in rice production are barnyardgrass (*Echinochloa crus-galli*), junglerice (*Echinochloa colona*), smallflower umbrella sedge (*Cyperus difformis*), purple nutsedge(*C. rotundus*), rice flatsedge (*C. iria*), goosegrass (*Eleusine indica*), lesser fimbristylis (*Fimbristylis littoralis*), saramollagrass (*Ischaemum rugosum*), pickerelweed (*Monochoria vaginalis*) and gooseweed (*Sphenochlea zeylanica*) (Holm et al 1977). In the world, the total estimated yield losses due to weeds is 15 - 66% (Gharde et al., 2018). In the US mid-south, specifically in Arkansas, the most problematic weeds in flooded rice include barnyardgrass, *Cyperus* spp., and weedy rice (*Oryza sativa* L.), while in furrow-irrigated rice barnyardgrass, Palmer amaranth (*Amaranths palmeri* S. Wats.), and *Cyperus* spp. are most problematic (Butts et al., 2022). The cost for weed management in Arkansas rice production is approximately \$266.40 ha⁻¹ (Butts et al., 2022).

Current herbicide options in rice

Herbicides are classified based on the modes of action, chemical families, time of application, selectivity, translocation, etc. (Duke, 1990; Varshney et al., 2012; Torrens, & Castellano, 2014). In US rice production, the most commonly used pre-emergence herbicides in rice production are Command (clomazone), Facet (quinclorac), and Prowl (pendimethalin). RiceBeaux (propanil + thiobencarb) is commonly used at early post-emergence (EPOST) timings. Permit plus (thifensulfuron + halosulfuron) or Permit (halosulfuron) or Gambit (halosulfuron + prosulfuron) are used on a few acres for broadleaves and yellow nutsedge control. FullPage rice was released by RiceTec in 2019 for use with Preface and PostscriptTM herbicides (Barber et al., 2020; Boyd 2019). The Preface and PostscriptTM herbicides belong to imidazolinone family of WSSA group 2 (ALS herbicides) and are widely applied to rice fields planted with Clearfield/FullPage[®] rice varieties. The use of Basagran (bentazon) has been rapidly increasing to manage the problematic sedges problem.

Herbicide-tolerant rice

Clearfield[®] Rice

Eradication of weedy rice in the rice production system is difficult due to its similar physiological and morphological characteristics to cultivated rice (Gealy et al., 2003). To resolve this problem, the first Clearfield® rice varieties CL121 and CL141 were commercialized in 2002

(Tan et al., 2005). Clearfield® rice is a non-transgenic, herbicide-tolerant rice with tolerance to the imidazolinone family of herbicides such as imazethapyr. Newpath (imazethapyr) is the primary herbicide labeled for Clearfield® rice to control weedy rice, barnyardgrass and other grass weeds, and some broadleaf weeds (Scott et al., 2013). As a result of its residual and foliar activity as well as its broad spectrum weed control, imazethapyr provides growers as a valuable tool for rice management. A major risk of growing Clearfield® rice is the persistent application of the imidazolinone herbicides (imazethapyr, imazamox, and imazapic) which leads to the evolution of resistant weedy rice (Sudianto et al., 2013). Currently, in Arkansas, there are six ALS-resistant problematic weeds in rice production: weedy rice (*Oryza sativa* L.), Pennsylvania smartweed (*Persicaria pensylvanica* (L.) M. Gómez), rice flatsedge, yellow nutsedge (*Cyperus esculents* L.), barnyardgrass, and Palmer amaranth (Heap 2022).

FullPageTM <u>Rice</u>

FullPageTM rice is a non-transgenic herbicide-tolerant rice technology developed through conventional breeding techniques. In 2019, FullPageTM rice was released by RiceTec for use with PrefaceTM (imazethapyr) and PostscriptTM (imazamox) herbicides (Barber et al., 2020). PrefaceTM and PostscriptTM are the only approved formulations of imazethapyr and imazamox that may be used with FullPageTM rice seed. PrefaceTM has both foliar and residual activity but PostscriptTM is only a foliar herbicide. Application of these herbicides with the other ALS inhibitors is also recommended.

Herbicide Safeners in rice

There are several methods to overcome herbicide phytotoxicity:1) the development of selective herbicides that are relatively safe for crops; 2) cultivating herbicide-resistant crops; and 3) using herbicide safeners (Hatzios et al., 1996; Abu-Qare et al., 2002; Tang et al., 2014; Gao et

al., 2019). Among these methods, herbicide safeners are widely used because of their effectiveness in overcoming herbicide phytotoxicity and are most economical.

Plant growth regulators

Plant growth regulators (PGRs) are organic compounds that influence a plant's physiological process at low concentrations. The Environment Protection Agency (EPA) defined PGR as any substance that increases or decreases plant growth and yield by altering its biological processes (Hopkins, 1999; Fishel, 2006). Growth regulator compounds produced naturally by plants are referred to as plant hormones but are known as PGRs when produced artificially to modify plant physiology and morphology (Avery, 1937). Plant growth regulators can be broadly divided into two groups: plant growth promoters (auxins, gibberellins, and cytokinins) and bio-inhibitors (ABA, and Methyl jasmonate). The PGRs are involved in various physiological processes such as cell division, cell enlargement, tropic growth, flowering, fruiting, seed formation, and senescence. Bio-inhibitors play an important role in stress response and are also involved in various developmental phases such as dormancy and abscission.

Biostimulants

The phrase biostimulant is increasingly used in scientific literature (Calvo et al., 2014; Halpern et al., 2015). The first definition of biostimulant was proposed by Kauffman et al. (2007) broadly describing it as materials that are plant growth promoters. According to the European Biostimulants Industry Council (EBIC) definition of biostimulants are plant chemical products and or microorganisms that, when used on plants or applied in the rhizosphere, promote natural process to enhance/benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, and crop quality" (EBIC, 2013). Biostimulants are natural in origin and can be used to improve plant growth and the quality of crops (Rouphael and Colla 2018). They are known to increase tolerance to

abiotic stress and to reduce the impact of harmful agrochemicals (Del Buono 2021; Gupta et al. 2021). Biostimulants are broadly classified into microbial and non-microbial types (Colla and Rouphael, 2015). There are two main categories of non-microbial biostimulants: organic biostimulants [seaweed extracts, protein hydrolysates, humic substances, smoke water, vermicompost leachate, chitosan and plant extracts] and inorganic biostimulants [Aluminum (Al), sodium (Na), selenium (Se), cobalt (Co), silicon (Si) and phosphite (H2PO2)] (Bhattacharyya et al., 2015; Canellas et al., 2015; du Jardin 2015). Commercially available biostimulants are mixtures of multiple bioactive compounds that can improve ion transport, nutrients update, nutrient efficiency, photosynthesis, phytohormones, or crop quality and yield and response to abiotic stress (Soppelsa et al., 2018; Wilson et al., 2015). Bray et al. (2000) reported that herbicide stress can reduce the average crop productivity by 10 - 15%. As a result, crop production becomes less profitable for growers. Biostimulants can contribute protection of plant health at various levels: physiological (via improved nutrient uptake and metabolism, antioxidant defense systems, and water relations; improved tolerance to reactive oxygen species; hormonal regulation; biochemical (via improved macromolecule biosynthesis, and mobilization of the food reserves); and genome modulating epigenetic change and chromatin function) (De Saegar et al., 2020).

This research is focused on Atonik, known as Chaperone (USA) or Asahi SL (Poland). Atonik is a synthetic biostimulant composed of three phenolic compounds: sodium paranitrophenolate PNP (0.3%), sodium ortho-nitrophenolate ONP (0.2%) and sodium 5nitroguaiacolate 5 NG (0.1%), and water that enhances growth and some essential metabolic processed of treated plants (Guo and Oosterhuis, 1995).

Goals and Objectives

The goal of this research is to explore the utility of Atonik in alleviating herbicide stress in rice, with the aim of safeguarding, if not improving rice yield.

Specific objectives of the research include:

- To determine if seed treatment with Atonik reduces injury from commonly used preplant herbicides.
- 2. To determine if seed treatment with Atonik can improve the performance of rice with commonly used pre-emergence herbicides.
- 3. To determine if seed treatment with Atonik can improve the performance of rice with commonly used delayed pre-emergence herbicides.
- 4. To evaluate the effect of Atonik on the crop safety of some foliar-applied rice herbicides.

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Chapter 2

Review of Literature

Rice herbicides

<u>Propanil</u>

Propanil is a photosystem II inhibitor (WSSA group 7) introduced in 1959 with excellent control grasses: barnyardgrass, broadleaf signalgrass (*Urochloa platyphylla*), crabgrass (*Digitaria sanguinalis*), fall panicum (*Panicum dichotomiflorum*); and broadleaf weeds: hemp sesbania (*Sesbania herbacea*), northern jointvetch (*Aeschynomena virginica*) (Scott 2017). Propanil is a broad-spectrum post-emergence herbicide that has been labeled for use in rice in 1961 (Senseman 2007), it has been used to control grasses and broadleaf weeds (Smith 1961, 1965; Smith and Hill 1990). Unlike other grasses, rice is tolerant to propanil (Baltazar and Smith 1994). Propanil is applied preflood (Smith and Hill, 1990). Propanil can cause rice injury up to 30% even when used at the labelled rate when environmental conditions (high temperature, high humidity, intense sunlight) favor maximum activity (Hoagland et al., 2004, Norsworthy et al., 2010, Osterholt et al., 2021). The fact that propanil has been used every cropping season for decades, resulted in the evolution of resistant weeds including barnyardgrass (*Echinochloa crus-galli*), junglerice (*Echinochloa colona*), and smallflower umbrella sedge (*Cyperus difformis*) (*Heap, I 2023*).

<u>Quinclorac</u>

Quinclorac (Facet 75DF, Facet L, Quinstar) is a synthetic auxinic herbicide that belongs to WSSA Group 4. In 1992, quinclorac was introduced to control broadleaf weeds and propanilresistant barnyardgrass in rice (Talbert & Burgos, 2007). Quinclorac can be applied preemergence, delayed pre-emergence, and post-emergence. It is the second most common preemergence herbicide used in Arkansas rice production (Norsworthy et al., 2007). However, quinclorac has little to no activity on sedges (Malik et al. 2010; Shaner 2014). Quinclorac causes approximately 5% injury to rice when applied at 420 g ai ha⁻¹ (Godwin et al., 2018). Quinclorac application on shallow-flooded fields have better weed control compared to deep-flooded rice field. This study was conducted in Limburgerhof, Germany on transplanted rice. The predominant weeds in this test were *Echinochloa* spp, and other weeds like *Sesbania exaltata, Aeschynomene* spp. *or Ipomea* spp. When this herbicide was applied foliar to rice in clay loam soil at 0.37 kg ai ha⁻¹, the rice was injured 10% but the yield was not affected (Kiessling et al., 1990).

<u>Clomazone</u>

An important option for weed control in rice is the application of herbicides prior to weed emergence (pre-emergence). Clomazone is an effective pre-emergence herbicide for Echinochloa species. Clomazone (Command 3ME) belongs to the WSSA group-13 isoxazolidinone chemical family and it inhibits the biosynthesis of diterpenes (Heap 2017). Clomazone is a 1-deoxy-Dxylulose 5-phosphate-inhibiting herbicide that acts by interfering with chloroplast development and reduces the accumulation of plastid pigments in susceptible weed species (Ferhatoglu and Barrett 2005). It was labeled for pre-emergence use in rice in 2000 (Norsworthy et al., 2007). Clomazone applied pre-emergence to rice at 0.39 ka ai ha⁻¹ on a coarse-textured soil controlled barnyardgrass 96% to 97%; and when applied post-emergence at 0.44 kg ai ha⁻¹ clomazone controlled two-leaf barnyardgrass 85% (Willingham et al. 2008). Clomazone can be applied to dry-seeded rice from 14 days before seeding to 7 days after seeding. Besides controlling barnyardgrass, clomazone also controls other annual grasses including Amazon sprangletop, crabgrass, fall panicum, and broadleaf signalgrass (Barber et al., 2020). Under certain conditions, clomazone can injure rice. Talbert et al.(1999) reported 3% bleaching of rice 'Lemont' when clomazone was applied pre-emergence at 0.45 kg ai ha⁻¹ in clay soil in Arkansas and the rice yield was 7% higher than check. Webster et al. (1999) reported 8 to 18% injury of 'Lemont' rice with clomazone applied as a pre-emergence. Clomazone was applied at the rate of 0.56 kg ai ha⁻¹ in clay loam soil and injury was evaluated 7 day after emergence. The rice yield was 4% higher than non-treated. Rice cultivars have differential tolerance to clomazone. A study conducted in California in 2002, using clomazone at 1.12 kg ai ha⁻¹ in clay-loam soil, showed that long-grain cultivars (e.g. L-206, Calmochi-101, A-202) were most tolerant (28% injury) than medium grain cultivars (e.g. M-401, M-402) which were injured 16% (Zhang et al., 2004). Despite the high injury of some cultivars, yield was not affected. The yield of long-grain cultivars was 1% lower than that of non-treated cultivars (8760 kg ha⁻¹), while the yield of medium-grain cultivars was 3% lower than that of non-treated cultivars (8760 kg ha⁻¹). Therefore, this level of injury did not affect yield. In 2004, the same research was conducted on short grain varieties (e.g. S-102, Calhikari-201, Calhikari-202, Koshihikari). Injury from clomazone ranged from 35 to 43% and the yield loss was higher, at 10% (Mudge et al., 2005).

Pendimethalin

Pendimethalin (Prowl, Prowl H_2O , etc) belongs to WSSA Group 3, or dinitroaniline herbicides (Devin et al., 1993). Pendimethalin acts by inhibiting microtubule formation in susceptible weed species (Shaner 2014). Pendimethalin damages the plant as it binds to tubulin molecules, thereby inhibiting cell division (Fennell et al., 2006). It is active only when applied to soil and is absorbed by germinating plant roots and coleoptiles, preventing emergence of susceptible weed species, death soon after emergence, or cessation of seedling growth due to lack of root development. Pendimethalin is effective on grass and small-seeded broadleaf weeds when applied prior to emergence. In rice, pendimethalin is applied delayed pre-emergence (two to three days after planting or post-emergence mixed with foliar herbicides before permanent flooding (Bond et al. 2009; Malik et al. 2010; Stauber et al. 1991). A study was conducted in Arkansas in 1991 and 1992, with pendimethalin, quinclorac, and fenoxaprop-p-ethyl applied delayed preemergence on silt loam soil with the objective of controlling herbicide-resistant barnyardgrass. In this test, pendimethalin controlled propanil-resistant barnyardgrass 12% better than quinclorac and fenoxaprop-p-ethyl (Baltazar et al., 1994). It has been reported that pendimethalin applied delayed pre-emergence at 1.8 kg ai ha⁻¹ in silt loam soil in Arkansas, controlled 74% of ALS-resistant barnyardgrass 21 days after treatment (DAT) (Norsworthy et al., 2014). Awan et al (2016) reported that application of pendimethalin pre-emergence on black soil at 2 kg ai ha⁻¹ reduced the stand of rice by 42% and the stem, leaf, and shoot biomass by 60%.

<u>Thiobencarb</u>

Thiobencarb (Bolero) belongs to WSSA Group - 8. Thiobencarb can be applied preplant, pre-emergence and delayed pre-emergence for controlling grasses such as amazon sprangletop, barnyardgrass and aquatic weeds [ducksalad (*Heteranthera limosa*), dayflower (*Commelina comunis*)]. Thiobencarb does not inhibit seed germination, but it inhibits the elongation of shoots from germinated seeds (Devine et al., 1993). The Herbicide Resistance Action Committee (HRAC) describes the target site for group-8 herbicides as lipid synthesis, but the specific mode of action is still unclear. These herbicides have practically no post-emergence activity but provides residual control of barnyardgrass and other another annual grasses in rice. Rice can be injured if thiobencarb is applied before the seed imbibition and the injury ranges between 15 to 30%. This experiment was conducted at the Rice Experiment Station, California, and it was conducted in clay loam soil. Thiobencarb was used at the rate of 1.777 kg ai ha⁻¹ (Fischer et al., 2004). Therefore Fischer et al., (2004) recommended to apply thiobencarb to a soil surface that has been sealed by rain or flushing to minimize injury and maximize activity. An experiment was conducted at the Rice Research

Experiment Station, Stuttgart, Arkansas, to determine the application timings of thiobencarb herbicide. Thiobencarb was used at a rate of 1.77 kg ai ha⁻¹. This study was conducted on a silt loam soil. This application caused 10% rice injury but did not affect the yield of rice (8299 kg ha⁻¹) (Scott et al., 2013). Herbicide efficacy and residual activity are reduced if the soil is dry (Hardke , 2014)

Florpyrauxifen-benzyl

Florpyrauxifen-benzyl (LoyantTM) is a synthetic auxin (WSSA Group 4). It is a postemergence, broad-spectrum herbicide that has activity on several weed species. The main symptoms of florpyrauxifen-benzyl injury are leaf malformation evidenced by rolled leaves and distorted stems, which may contribute to the reduction in biomass. Stalk strength is an important factor in rice lodging resistance (Kashiwagi et al., 2008; Zuber et al., 2001), and damaged stems could cause rice to lodge.

Florpyrauxifen-benzyl controls many problematic weeds in rice production, including hemp sesbania 98%, yellow nutsedge 93%, and barnyardgrass 97% when applied at the recommended field rate (Miller and Norsworthy, 2018). Miller and Norsworthy (2018) reported that florpyrauxifen-benzyl does not have residual activity and should be applied with a residual herbicide for control of troublesome weeds such as barnyardgrass, northern jointvetch, sprangletop, palmer amaranth and other broadleaf weeds.

Miller et al., (2018b) reported that florpyrauxifen-benzyl is expected to exhibit optimal weed control under a flooded system; however, some activity has been shown in dryland cropping systems as well. Thus, Miller et al. (2018b) evaluated florpyrauxifen-benzyl for weed control in the absence of a permanent flood. The above experiment was conducted in a greenhouse in Fayetteville, Arkansas, with the purpose of determining florpyrauxifen-benzyl translocation and

metabolism in three weed species (barnyardgrass, yellow nutsedge, and hemp sesbania). The herbicide was applied at 3 to 4 leaf stage of weeds at the rate of 30 g ai ha⁻¹.

Another study was conducted by Wright et al., (2018) to determine rice cultivar tolerance to florpyrauxifen-benzyl. The experiment was conducted in Arkansas in 2016, results showed that; a long-grain inbred variety 'CL111', medium grain varieties 'CL272' and long grain hybrid 'CLXL745' are more sensitive to florpyrauxifen-benzyl herbicide when compared to non-treated. The use rate of herbicide was 30 and 60 g ae ha⁻¹; and applied at three-leaf stage. They reported that CL272 and CLXL745 are sensitive to sequential applications of florpyrauxifen-benzyl. CLXL745, is especially sensitive, and caution should be used when applying florpyrauxifenbenzyl to this rice cultivar. However,CLL111 has exhibited sufficient tolerance to florpyrauxifenbenzyl with only 10% injury visible injury and no impact on yield.

Another study was conducted by the Wight et al., 2018) in the greenhouse to evaluate the effect of florpyrauxifen-benzyl rate and growth stage at the time of application. They evaluated three cultivars ('CL111, 'CL272', and 'CLXL745'), two herbicide rates (30 and 60 g ae ha⁻¹), and three rice growth stages (1-,3- and 5-). reported that, the highest injury of 17% with CL111 was observed when herbicide was applied to 1-leaf rice, averaged across rates. Additionally, more reduction in plant height, tillers production, and biomass observed when the herbicide is applied at the 1-leaf stage in CL111 cultivar when compared to other cultivars.

<u>Triclopyr</u>

Triclopyr (Grandstand), a synthetic auxin herbicide that belongs to WSSA Group - 4 is used post-emergence to control common chickweed (*Stellaria media*), henbit (*Lamium amplexicaule*), knotweeds (*Fallopia japonica*), White Clovers (*Trifolium repens* L. TRFRE), dandelion (*Taraxacum officinale*). Besides these species, triclopyr is also effective in controlling annual broadleaf weeds in rice such as Indian jointvetch (*Aeschynomene indica*), northern jointvetch (*Aeschynomene virginica*), palmleaf morninglory (*Ipomoea wrightii*) and Texasweed (*Caperonia palustris*) (Anonymous 2011). Generally, this herbicide is safe for rice, except for rice that is flooded within 36 hours of application. The injury to rice was less than 20% when triclopyr was applied at post-flood stage (Willingham et al., 2008).

Pantone and Baker (1992) examined the tolerance of three rice varieties ('Lemont', 'Mars', 'Tebonnet') to triclopyr. In this study, triclopyr was applied at two rates at three growth stages (2to 3-leaf stage, 4- to 5- leaf stage, and panicle initiation). When triclopyr was applied to 4- to 5leaf rice at 800 g ae ha⁻¹, Lemont was injured 40%, while Mars and Tebonnet were injured less than 20% (Pantone and Baker 1992). However, when triclopyr was applied at 400 g ae ha⁻¹, injury to Lemont was less than 20% and injury to the other varieties was less than 10%. This study also demonstrates how crop injury can sometimes influence yield. Yield of Lemont was reduced over 30% when 800 g ae ha⁻¹ triclopyr was applied to 2- to 3-leaf rice. However, yield was reduced only 5% when plants were sprayed with the lower rate. For all cultivars evaluated in this experiment, triclopyr applied at 800 g ae ha⁻¹ to small rice plants caused the most reductions in yield (Pantone and Baker 1992). This study demonstrates injury and crop yield can be affected by several factors, including herbicide rate, crop growth stage, and cultivar. Because of the potential injury, triclopyr is recommended only on certain cultivars (Anonymous 2002).

Cyhalofop-butyl and Fenoxaprop-p-ethyl

Cyhalofop-butyl (Clincher) and fenoxaprop-p-ethyl (Ricestar HT) belong to the ACCase-inhibitor WSSA Group-1 herbicides. Herbicides in this group inhibit the ACCase enzyme, preventing fatty acid synthesis, resulting in a limited production of phospholipids required for cell growth. In broadleaf species, the enzyme is insensitive, hence these herbicides are effective only on grasses

(Konishi et al., 1994). In Arkansas rice production, only cyhalofop-butyl and fenoxaprop-p-ethyl are registered ACCase herbicides to conventional rice varieties (Scott et al., 2017). Both herbicides are selective to rice (Barber et al., 2020). Quizalofop (Provisia) is registered for ACCase-inhibiotr herbicides are effective on barnyardgrass, broadleaf signalgrass (*Urochloa platyphylla*), Amazon sprangletop (*Leptochloa panicoides*) as well as other grass weeds (Scott et al. 2015). These herbicides can be applied post-emergence at three leaf-stage and after flooding. Cyhalofop-butyl is effective against barnyardgrass for up to ten days after flood irrigation.

A study was conducted in Arkansas to control herbicide-resistant barnyardgrass in 2010. In this study both herbicides were applied at 3- to 4- leaf stage (Norsworthy et al., 2012). They reported that the application of fenoxaprop-p-ethyl at a rate of 120 g ai ha⁻¹ to 3- to 4-leaf stage of barnyardgrass controlled ALS-susceptible barnyardgrass up to 99% compared to nontreated grass. In addition, cyhalofop-butyl herbicide was applied at a rate of 314 grams per hectare ai ha-1 to control both resistant and susceptible barnyardgrass genotypes. There were no significant differences between resistant and susceptible genotypes in control levels. Generally, barnyardgrass cannot be completely controlled with a single application of cyhalofop-butyl (Buehring et al. 2006). The barnyardgrass plants were not flooded in Buehring's experiment, which is not typical in Arkansas rice cultivation, and the cyhalofop-butyl label (Anonymous 2012) indicates that the herbicide is most effective on flooded rice. ACCase-inhibiting herbicides are effective on barnyardgrass with resistance to other herbicides with different modes of action such as propanil, quinclorac, or ALC inhibitors.

Fenoxaprop-p-ethyl is injurious to various rice cultivars, with the level of injury dependent on the stage of growth and environmental conditions at the time of application (Griffin and Baker 1990, Snipes and Street 1987; Snipes et al., 1987). This study

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A study was conducted in 1987 and 1988 to test the tolerance of rice cultivars to fenoxaprop-p-ethyl at the Rice Research Station, Louisiana. The rice cultivars tested were a medium grain cultivar 'Mars' and two long-grain cultivars 'Lemont' and 'Tebonnet'. Fenoxaprop-p-ethyl was applied at 0.336 kg ai ha-1 at preflood (PRF) and postflood of rice (POF). It has been reported that Fenoxaprop applied both PRF and POF reduced yields of Mars rice. Lemont and Tebonnet were most susceptible to fenoxaprop when applied POF. Additionally, they also reported that application of fenoxaprop-p-ethyl PRF on rice cause 10% more injury and 8% less yield than application at POF. ACCase inhibitors play an important role in the control of propanil-, quinclorac-, and/or ALS-resistant barnyardgrass populations.

<u>2,4–D</u>

2,4-dichlorophenoxy acetic acid belongs to the phenoxy family of Group-4 herbicides. It was commercialized in 1940s and has been crucial tool for weed control across many crop and noncrop situations. It has been reported that 2,4-D can injure rice when applied pre-emergence (Peterson et al., 2016). The injury on rice was 43% and the rice yield was reduced by 28% compared to non-treated control. Also reported that there was no injury when 2,4-D was applied 7 or more days before planting. Both studies were conducted in silt loam soil in Northeast Research Station, Louisiana (Jordan et al., 1997). 2,4-D is currently the standard for broadleaf weed control on levees. However its use in key rice producing counties is restricted due to the proximity of cotton (*Gossypium hirsutum* L.). Cotton is extremely sensitive to 2,4-D so in many counties, 2,4-D cannot be applied without a permit (ASPB 2002; Carns and Goodman 1956). Thus, options to control Palmer amaranth and other primary broadleaf weeds in furrow-irrigated rice production and on rice levees are limited, making broadleaf weed control in these environments challenging. A study was conducted in Arkansas in silt loam soil to evaluate florpyrauxifen-benzyl and 2,4-D weed control programs on rice levees (Wright et al., 2021). 2,4-D was applied at 1600 g ae ha⁻¹ and florpyrauxifen-benzyl was at 30 g ae ha⁻¹ 3-to 4-leaf rice. They reported that rice injury from 2,4-D was less than 8%.

<u>Saflufenacil</u>

The herbicide saflufenacil is relatively new and was previously used only for burndown applications prior to planting. Saflufenacil can be used as a pre-plant and pre-emergence herbicide in rice production. However, saflufenacil is now labeled for use as a post-emergence herbicide in rice at an application rate of 0.0247 kg ha⁻¹. Since saflufenacil is a relatively new herbicide in rice, research is ongoing to determine the most effective method of application. Saflufenacil could injure rice 24%, but rice recovered from the injury and there was no yield loss compared to the nontreated control (Dickson et al. 2014). As a result of a reduction in weed pressure, some plots treated with saflufenacil produced higher yields than those not treated. The results of a study conducted by Camargo et al. (2012) were similar to those found by other researchers. A significant degree of damage (15%) was also observed after applying saflufenacil post-emergence; however, yield was not affected (Camargo et al. 2012). Montgomery et al. (2014) reported that hybrid CLXL745 and medium grain varieties 'Caffey' and 'Cl261' were more sensitive to saflufenacil (15 to 18% injury, with 5% less yield) than two long grain cultivars CL111 and CL272. Another research has also shown that saflufenacil can reduce rice yield (Fickett et al., 2012). This study was conducted in black alluvial soil with the objective of managing Indian jointvetch and hemp sesbania in rice production. The application rate was 0.0237 kg ha⁻¹. Fickett and colleagues reported that saflufenacil caused 12% injury to rice and 8% reduction in yield compared to nontreated control. Generally, saflufenacil provides excellent broadleaf weed control; however, it causes significant leaf necrosis following application.

<u>Mesotrione</u>

Mesotrione (Callisto) is a selective herbicide, that belongs to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors(WSSA group - 27), which can be used pre-emergence and postemergence on most labeled crops such as corn, and soybean (Senseman 2007). It was introduced in 2001 in the US markets. Chemically, mesotrione [2-(4-mesyl-2-nitrobenzoyl) cyclohexane-1, 3-dione] belongs to the triketone family (Cornes 2005). Mesotrione is typically used for broadleaf control; however, it also controls certain annual grasses such as barnyardgrass, smooth crabgrass [*Digitaria ischaemum* (Schreb.)], fall panicum, and large crabgrass (Soltani et al. 2011).

In recent years, benzobicyclon a triketone family herbicide, has recently been developed and commercialized for rice (Davis et al., 2014; Van Almsick, 2009). Benzobicyclon is an excellent herbicide for controlling barnyardgrass, Amazon sprangletop, and other rice weeds in flooded ecosystems (Davis et al., 2014). There is, however, evidence that HPPD- inhibiting herbicides, including mesotrione and benzobicyclon, cause severe injury to multiple rice varieties, with *japonica*-type rice being more tolerant than *indica*-type rice (Kwon et al., 2012). It is essential to further research other HPPD-inhibiting herbicides, such as mesotrione, for utility in rice production.

Applications of Atonik in crop production

In early to mid-1990s, Atonik has been applied on different crops in more than 20 countries including cotton (*Gossypium hirsutum*), rice and soybean (*Glycine max*) as ARYSTA-EXP_NP321 (Asahi Co., Ltd). The EPA has registered ARYSTA_Exp_NP321 under the trade name Chaperone. The compound has been characterized as a protein transport enhancer (Bynum et al., 2007). Trade names of Atonik are:

- GWN10598 and Asahi SL I used in rice. It can be applied as a seed and foliar treatment (Borowski et al., 2009).
- 2. Chaperone cotton seed and foliar treatment (Carlos J Fernandez, 2007).
- 3. AEGISTMESR cotton foliar treatment (Glwen K and Thompson. L, 2006).

Atonik has been used in various crops including cotton and tomato (Djanaguiraman et al., 2005), bean (*Phaseolus vulgaris* L) (Kocira et al., 2013), soybean (Kozak et al., 2008), carrot (Kwiatkowski et al., 2013), maize (Przybysz et al., 2014), and other crops. Research was conducted by Djanaguiraman et al., (2006) at Tamil Nadu Agricultural University, Coimbatore, India with the objective of studying the effects of Atonik seed treatment on cotton and tomato seedling physiology. The use rate of Atonik ranged between 0 to 6 ppm at increments of 1 ppm. They reported that, in cotton, Atonik seed treatment at 6 ppm increased seed germination, 15% and dry matter 43% compared to the check. While in tomatoes Atonik seed treatment increased seed germination 30% and dry matter 40%.

In another study, Atonik was applied foliarl(2-leaf stage) at a rate of 5 ppm to determine how Atonik affected carrot root yield and root quality, including dry matter, carotenoids, and total sugars (Kwiatkowski et al., 2013).This experiment was conducted in Fajslawice, Lublin, Poland. Atonik was used at 5 ppm. The authors reported that Atonik increased the total carotenoid content in carrot by 6%. Also, it was shown that it positively affected carrot root production (4%), dry matter, carotenoids, L-ascorbic acid content, and total sugars.

Szparaga et al., (2019) conducted an experiment to determine the effect of Atonik on common bean (*Phaseolus vulgaris*) in Poland. They reported that application of Atonik has improved the biometric traits (seed number, number of pods and seed yield) by 3%. In this study, Atonik was applied at three-leaf stage at 0.2% v/v.

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Herbicide (Reference)	Year	Rate (kg ai ha-1)	Location	Soil type	Variety	Application timings	Visible response	Yield reduction
Quinclorac (Norsworthy et al., 2017)	2007	0.56	Stoneville, Mississippi	Sharkey clay	CL161,Bowman, Cheniere Cocodrie, XL723	2 and 4 WAF ^a	No injury; delayed maturity of Cheniere and XL723	Cheniere = 3%, XL273 = 5%
Triclopyr (Jacoby et al., 1982)	1982	0.30 - 0.60	Stuttgart, Arkansas	Silt loam	Bond	Early - tillering, jointing, early-boot, and late- boot	Injury = 31 and 36% respectively ay early- tillering and late boot timing.	Late boot =18%;other timings, none
Florpyrauxifen-benzyl (Miller et al., 2018)	2018	0.30 - 0.60	Stuttgart, Arkansas	Silt loam	CL111, CL272, and CLXL745	Three-leaf stage	10 - 30%	CLXL745 =19%, CL272 = 15%, CL111= 10%
Clomazone (Webster et al., 1999)	1999	0.34 - 0.67	Stuttgart, Arkansas	Silt loam	Ahrent, Bengal, Cocodrie, Cypres, and RU961096	PRE ^c	RU961096 = 30 to 40% chlorosis	RU961096 = 0 to 5% (not- significant)
Thiobencarb (Fischer et al., 2004)	2002	1.68 - 3.36	Crowley, Louisiana State University	Clay loam soil	CL1161, CL111 and CL151	2 DAP ^b	Injury = 13 to 15%; highest with CL111	CL111 = 4% (not-significant).

 Table 1: Rice response to some key herbicides across various locations in the US mid-south

Table 1 (Cont.)

Herbicide (Reference)	Year	Rate (kg ai ha-1)	Location	Soil type	Variety	Application timings	Visible response	Yield reduction
Propanil (Vories et al., 2002)	2002	0.56 - 1.12	Poinsette, Arkansas	Silt loam	Tebonnet	Pre and three-leaf stage	Injury = 8 to 30 %	15 to 20%
Cyhalofop- butyl and Fenoxaprop-p- ethyl (Griffin and Baker., 1990)	2011	0.33	Crowley, Louisiana	Silt loam	Lemont, Mars and Tebonnet	three-leaf stage	Injury = 10 to 12 %	Yield loss = 8% compared to check.

Abbreviations: WAF: application at 2 and 4 weeks after flowering; DAP: days after planting; PRE: pre-emergece.

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Chapter 3

Rice Seed Treatment with Atonik and Response to Soil-applied Herbicides

Srikanth Kumar Karaikal¹, Isabel Schlegel Werle¹, Matheus Machado Noguera¹, Gustavo Henrique Bessa de Lima¹, and Nilda Roma-Burgos²

 ¹Graduate Research Assistant, University of Arkansas, Department of Crop, Soil and Environmental Sciences, Fayetteville, AR, USA
 ²Professor, University of Arkansas, Department of Crop, Soil and Environmental Sciences, Fayetteville, AR, USA

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Abstract

Atonik is a plant growth regulator that could reduce abiotic stress including herbicide stress. Previous research explored the potential of Atonik to reduce rice injury from herbicides. Multiple field experiments were conducted in the summer of 2021 at the Southeast Research and Extension Center, Rohwer, Arkansas and greenhouse experiments were conducted in the spring of 2022 at Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR. Field experiments were conducted in silt loam and clay loam soil. Field study aimed to determine if Atonik seed treatment at 0.225 ml kg⁻¹ seed would improve the performance of rice with various preplant herbicides. The herbicides tested were fluridone (Brake), fluridone + diuron (Brake + Direx) metribuzin + chlorimuron (Canopy), diuron, oxyfluorfen (Goal), triclopyr + clopyralid (GrandStand + Stinger), linuron (Linex), saflufenacil (Sharpen), chlorimuron + flumioxazin + metribuzin (Trivence), flumioxazin (Valor WG), 2,4 - D (Weedar 64), and 2,4 - D + thiobencarb (Bolero). Saflufenacil was used as a reference herbicide treatment. All herbicides were applied at label rates to 'CLL-16' rice at 30 days prior to planting. Plots with Atonik seed treatment were also sprayed with 0.225 % v/v Atonik at 3-leaf stage of rice. In both soil types, injury was minimal (generally 10%), except with metribuzin + chlorimuron and chlorimuron + flumioxazin + metribuzin (> 60%) injury. Seed treatment with Atonik did not reduce injury. Atonik seed treatment generally did not benefit rice yield in clay loam soil; however, Atonik seed treatment increased rice yield triclopyr + clopyralid 869 kg ha⁻¹ relative to rice without seed treatment. In silt loam soil, yield increased numerically by 1043 to 1882 kg ha⁻¹ in 9 of 12 treatments (mean increase = 1294 kg ha⁻¹). The second field study consisted of Atonik seed treatment at 0, 0.375, 0.75, 1.5, and 3.0 ml kg⁻¹ seed and combinations of pre-emergence herbicides. These include quinclorac + thiobencarb, quinclorac + pendimethalin, quinclorac + clomazone, and non-treated. Quinclorac +

pendimethalin caused the highest injury (57%) to rice, averaged across Atonik concentrations. Seed treatments did not improve rice yield. In the greenhouse, seven rates of Atonik seed treatment: 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml kg⁻¹ seed, with four herbicide treatments. Overall, the highest biomass was produced with 2.5 ml kg⁻¹ seed treatment without herbicides in the greenhouse. The last field study consisted of the same treatments, but with herbicides applied 2 days after planting (or delayed pre-emergence). Overall, in both studies, the highest yield of 14246 kg ha⁻¹ was produced when rice seeds were treated with 0.375 ml Atonik a quinclorac + pendimethalin herbicide and no seed treatment). The delayed pre-emergence study was also conducted in the greenhouse in 2022. Overall, the highest biomass was produced when quinclorac + pendimethalin was applied to rice seeds treated with 2.0 ml Atonik. In conclusion, Atonik seed treatment may improve rice yield with some soil-applied herbicides, but the benefit is highly variable.

Nomenclature: flumioxazin, chlorimuron, Canopy, Trivence, quinclorac, clomazone, thiobencarb, pendimethalin, rice, *Oryza sativa* L.

Key words: herbicide, rice injury, yield.

Introduction

Nearly half of the world's population depends on rice (*Oryza sativa* L.), one of the major food crops, for both food and money. In 2021, the United States produced 6.885 million metric tons of milled rice, with the majority of this production taking place in four regions (Arkansas, Mississippi Delta, Gulf Coast, Sacramento Valley California) of the United States [1]. Approximately 2,534,000 ha of rice were planted across the U.S. in 2021, with more than 70%

being long grain crop [2]. In Arkansas, rice production contributes more than US\$1 billion to the economy annually [3]. The most limiting factor for rice production is weed control because weed competition with rice could reduce yield by upto 5% [4]. The most problematic weeds in Arkansas rice production are barnyardgrass (Echinochloa crus-galli L. P. Beauv), sedges (Cyperus spp.), Amazon sprangletop (Leptochloa panicoides A.S. Hitchc), and weedy rice (Oryza sativa) [5]. Barnyardgrass can cause more than 55% grain yield reduction [6] while weedy rice can cause a potential loss of up to 72% if left uncontrolled throughout out the season. Soil-applied herbicide options in the U.S. prior to weed germination in rice include pendimethalin, quinclorac, clomazone, imazethapyr, thiobencarb and saflufenacil [7]. These herbicides could occasionally cause some injury to rice including loss of stand, stunting of seedlings, or bleaching in the case of clomazone. For example, clomazone can cause rice injury from 10 to 18% when applied at labeled rate [8], quinclorac causes approximately 5% injury to rice when applied at 420 g ai ha⁻¹ [9], and pendimethalin causes a loss of 42% crop stand, as well as decrease in stem, leaf, and shoot biomass by 60% when applied at 2 kg ai ha⁻¹ [10]. The level of injury is generally minimal but could be substantial during unfavorable environmental conditions such as cold and wet weather early in the season and could be exacerbated by soil-related pest problems or micronutrient problems. This occasional injury from herbicides could cause 'hidden' yield loss and maybe be alleviated by using products that could mitigate the effect of environmental or herbicidal stress. One such products is Atonik, also known as Chaperone (USA) or Asahi (Poland), which was registered in 2000 [11]. Atonik is a synthetic biostimulant composed of three phenolic compounds: sodium paranitrophenolate PNP (0.3%), sodium ortho-nitrophenolate ONP (0.2%), and sodium 5nitroguaiacolate 5NG (0.1%), and water. Atonik has been widely used in various crops including cotton [12], bean [13] soybean [14], carrot [15], maize [16], and other crops. However, it has never

been used in rice production. Researchers reported that Atonik has a positive effect on yield in most important crops (rice, cotton, tomato)[17–19]. Foliar application of Atonik increases the inhibition of IAA oxidase, which results in greater activity of naturally synthesized auxins [16], and increases in cytoplasm streaming [20], photosynthesis and transpiration rate [21], and plant nutrients uptake [22]. One study showed that Atonik-treated maize plants produce more nitrate reductase enzyme and, therefore, could contribute positively to nitrogen metabolism [16,23,24]. How Atonik improves crop performance is not thoroughly understood. Most abiotic stresses cause excessive levels of reactive oxygen species (ROS), which result in debilitating oxidative stress [25]. It has been reported that application of Atonik reduces the level of oxidative stress by increasing i) the activity of antioxidants ascorbate peroxidase, catalase and gluthathione reductase, and ii) the total antioxidative capacity [17,26]. In addition, Atonik also affects metabolite production (proline and polyols) involved in anti-stress mechanisms.

The long-term intensive use of rice herbicides has resulted in the evolution of several herbicide-resistant grasses and sedges and the evolution of resistance to multiple herbicides in one species such as the case of *Echinochloa* spp. There is continuing need to increase the diversity of herbicide choices in rice, especially herbicides for preplant applications, to reduce weed population size in-season. Several residual herbicides have excellent activity on grass weeds but cause high injury to rice. The use of Atonik as seed treatment may reduce rice injury from certain soil-applied herbicides and may allow the use of some otherwise injurious herbicides as preplant weed management option.

Preliminary field studies were conducted in 2018 and 2019 with three factors: Atonik seed treatment, herbicide, and application timings. Overall, in the pre-emergence experiment, seed treatment with 0.75 ml kg⁻¹ seeds and quinclorac + pendimethalin produced 1,100 kg ha⁻¹ higher

yield than nontreated check in 2018, but not in 2019 (Appendix Table 1). In clay loam soil, seed treatment with Atonik from 0.5 to 1.5 ml kg⁻¹ seed produced 550 kg ha⁻¹ higher yield compared to no seed treatment when quinclorac + pendimethalin was applied pre-emergence. In silt loam soil, the yield was increased by 743 kg ha⁻¹ with the same Atonik rates and herbicide combinations.

Experiments were conducted on rice to 1) determine the optimum concentration of Atonik seed treatment with preplant herbicides; 2) determine the optimum concentration of Atonik for rice seed treatment to improve crop safety with pre-emergence herbicides; and 3) determine the optimum concentration of Atonik for rice seed treatment to improve crop safety with delayed pre-emergence herbicides.

Materials and Methods

Study A: Atonik seed treatment with preplant herbicides

Study A.1. and A.2. Response of rice to Atonik seed treatment and preplant herbicides in two soil types in the field.

Two field experiments were conducted at the Southeast Research and Extension Center, Rohwer, AR in 2021 in silt loam (block W1C) and clay loam soil (block BW6A). The chemical properties of the two types of soil are listed in Table 1.

This study consisted of two levels of Atonik seed treatment including 0 and 0.225 % v/wt; and 12 levels of herbicides, including fluridone (Brake), fluridone + diuron (Brake + Direx) metribuzin + chlorimuron (Canopy), diuron, oxyfluorfen (Goal), triclopyr + clopyralid (GrandStand + Stinger), linuron (Linex), saflufenacil (Sharpen), chlorimuron + flumioxazin + metribuzin (Trivence), flumioxazin (Valor WG), 2,4 - D (Weedar 64), and 2,4 - D + thiobencarb as the main factor (Table 2). The experimental design was a split-plot randomized complete block with four replications with herbicide as whole plot and Atonik concentrations as subplot. The plot size was 1.5 m X 4.8 m with 9 rows of rice spaced and 15.24 cm between rows. The herbicides were applied 30 days prior to planting at labeled rates. Prior to planting: Clearfield (CLL16) rice seeds were treated with Atonik either 0 or 0.225 % v/wt of rice seed. After treatment, the seeds were incubated for 2 hr in Ziplock bags and dried overnight at room temperature.

The rice seeds were drilled-seeded on May 17th, 2021 at 56 kg ha⁻¹, which is in the normal seeding range for drill-seeded rice in the US. At 1 week after planting (WAP), the test area was sprayed with paraquat and glyphosate at 1.276 and 1.348 kg ai ha⁻¹ respectively, to control barnyardgrass (*Echinochloa crus-galli*) and broadleaf signalgrass (*Urochloa platyphylla*). Other maintenance herbicides were applied as needed during the growing season (Appendix table 2).

Data collected included crop stand from two 0.5 m length of row; injury at 2, 4, and 6 WAT (weeks after treatment); rice height; number of tillers and panicles; and rough rice yield. Rice injury was evaluated on a 0 to 100 scale, with 0 means no injury and 100 is dead [27]. Rice height was measured from 6 plants plot⁻¹. Rough rice yield and moisture content were recorded per plot and yield was adjusted to 12% grain moisture.

In this study, crop injury data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, N.C.), and means were separated using Tukey's protected least significant difference (LSD) (P 0.05). In contrast, plant height, panicle count, and yield were analyzed using the fit-model platform, JMP Pro 16.1(SAS Institute Inc., Cary, NC). In the analysis, herbicide, Atonik seed treatment, and their interaction were analyzed as fixed effects. The block, block x herbicide, and block x Atonik, were included in the model as random effects. All data were subjected to analysis of variance (ANOVA), and treatment means were separated by Tukey Kramer LSD test at $\alpha = 0.05$. All quantitative data were reported as actual values, with crop injury data being reported relative to non-treated control.

Study B: Atonik seed treatment and pre-emergence herbicides

Study B.1. Response of rice to Atonik and pre-emergence herbicides in the greenhouse

Greenhouse experiments were conducted on January and March, 2022 at the Milo J Shult Agricultural Research and Extension Center (SAREC), Fayetteville, AR (36° 5'55.213'' N, 94°10'43.038''W). Silt-loam soil was collected at SAREC, Fayetteville, AR. A composite sample of this soil was sent to the University of Arkansas Diagnostics Laboratory in Fayetteville, Arkansas for analysis of chemical and physical properties (Table 1). The soil was dried in the greenhouse at 35 °C for 2 weeks and 4.5 kg was added to a 1-gal bucket (base diameter - 19.05 cm, height - 18.7 cm, top diameter - 20.32 cm). Soil moisture was calculated to determine how much water is required to maintain 100% field capacity of the soil.

Mositure (%) =
$$\frac{\text{Fresh weight (Dry weight)}}{\text{Total weight}} X 100$$

The greenhouse experiment was a split-plot randomized complete block design with four replications with herbicide as main plot and Atonik concentration as the split-plot. The experiment consisted of 28 treatments including non-treated checks. Seeds (400-g batches) of 'RT7321FP' rice (FullpageTM Rice Tec, Alvin, Texas) were treated with seven concentrations of Atonik (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ml kg⁻¹ seed). The treated seeds were incubated in Ziplock bags for 2 hr, and air-dried in a dishpan overnight at room temperature. The rice was planted at a density of 14 seeds bucket⁻¹ into soil with a moisture content of 8% field capacity at a depth of 2.5 cm. The herbicides were applied immediately after planting, in a spray chamber fitted with a motorized boom with two flat-fan 1100067 nozzles (TeeJet, Glendale Heights, IL) calibrated to deliver 187 L ha⁻¹. The herbicide treatments, trade names, application rates, application timings, and manufacturers are provided in Table 3.

After herbicide application, the buckets were moved to a greenhouse maintained at 32/25 °C ± 3°C day/night temperature with a 16-hour photoperiod. The buckets were watered to 100% field capacity and maintained at this moisture level. Ten days after planting, seedlings were counted and thinned to 4 bucket⁻¹. The buckets were flooded (2.5 cm above the soil), and urea (168 kg ha⁻¹) was applied in three splits (30, 60 and 90 days after planting). Potassium (56 kg ha⁻¹) was applied before planting. Fertilizer was applied based on the soil analysis report and the recommendation for rice. This experiment was conducted twice.

Data collection and analysis

Emerged seedlings were counted 1 WAT. Rice injury was evaluated visually, and rice height was measured at 2, 4, and 6 WAT. At 6 WAT, 3 plants bucket⁻¹ were cut at the soil surface, dried at 60 °C for 4 to 5 days, and weighed. The remaining plant was cultured until maturity and grain yield per plant was recorded.

In this study, crop injury data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC), and means were separated using Tukey's protected least significant difference (LSD) at $\alpha = 0.05$. Plant height and biomass data were analyzed using the fit-model platform, JMP Pro 16.1(SAS Institute Inc., Cary, NC). In the analysis, herbicide, Atonik seed treatment, and their interactions were analyzed as fixed effects. The block, block x herbicide, and block x Atonik, were included in the model as random effects. All data were subjected to analysis of variance (ANOVA), and treatment means were separated by Tukey Kramer LSD test at $\alpha = 0.05$. All quantitative data were reported as actual values. Crop injury was evaluated relative to the non-treated controls.

Study B: Atonik seed treatment with pre-emergence herbicides

Study B.2. and B.3. Response of rice to Atonik seed treatment and pre-emergence herbicides in clay and silt loam soil in the field

Field trials were conducted at the Rohwer Research and Extension Center (37°12' 49.272" S, 142° 34' 4.758" E) Rohwer, Arkansas in the summer of 2021 in silt loam (block W1C) and clay loam soil (block BW6A). Soil samples were collected from the experimental site and sent to the Agricultural Diagnostic Laboratory at the University of Arkansas, Fayetteville, AR for analysis (Table 1). Soil texture was analyzed using the hydrometer method. The experimental design was a split-plot randomized complete block with 4 replications with herbicide as mainplot (4 levels) and Atonik concentration as subplot (5 levels). Details about the herbicide treatments are listed in Table 3.

The experimental site was tilled conventionally. The whole experimental area was oversprayed with paraquat and glyphosate at the rate of 1.276 and 1.348 kg ai ha⁻¹ 1 week before planting foliar herbicides were broadcast-applied to the whole test at 2 leaf stage of rice and later to control the remaining weeds (Appendix Table 3). The soil test did not warrant preplant application of nitrogen. Urea (46-0-0) was applied by airplane in two splits: 123 kg ha⁻¹ before flooding (4 WAP) and 335 kg ha⁻¹ 8 WAP.

Hybrid rice 'RT7321FP' was planted at 72 seeds m⁻¹ of row on May 25th, 2021, with seed treatment of 0, 0.375, 0.75, 1.5, and 3.0 ml Atonik kg⁻¹ seed. Each plot was 1.5 m wide and 4.8 m long, with 8 rows of rice 19 cm apart. The distance between two adjacent plots was 1.5 m and 0.9 m between two blocks. Herbicides were applied immediately after planting, using a CO₂-pressurized backpack sprayer attached to a hand-held spray boom with three 8002 flat fan nozzles (Teejet, Glendale Heights, IL) spaced 45.72 cm apart calibrated to deliver 187 L ha⁻¹at 206 kPa⁻¹.

Data collection and analysis:

Rice seedlings were counted at 2 WAT from 0.5-m length of row at two locations per plot at. Rice injury was visually evaluated relative to the nontreated control 2, 4 and 6 weeks after treatment +/- 2 days. The number of tillers m⁻¹ and the number of panicles m⁻¹ were counted at maturity. Rice was harvested using a small plot combine that records yield per plot and grain moisture. Rice grain yield was expressed in kg ha⁻¹ adjusted to 12% moisture.

In this study, crop injury data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC), and means were separated using Tukey's protected least significant difference (LSD) (P 0.05). Plant height, panicle count, and yield were analyzed using the fit-model platform, JMP Pro 16.1(SAS Institute Inc., Cary, NC). In the analysis, herbicide, Atonik seed treatment, and their interaction were analyzed as fixed effects. Block, block x herbicide, and block x Atonik, were included in the model as random effects. All data were subjected to analysis of variance (ANOVA), and treatment means were separated by Tukey Kramer LSD test at $\alpha = 0.05$. All quantitative data were reported as actual values. Crop injury was evaluated relative to the non-treated controls.

Study C: Atonik seed treatment with delayed pre-emergence herbicides

C.1. Rice Response to Atonik seed treatment and delayed pre-emergence herbicides in the greenhouse

Greenhouse experiments were conducted in February and April 2022 at the Milo J Shult Agricultural Research and Extension Center (SAREC), Fayetteville, AR (36° 5'55.213'' N, 94°10'43.038''W). Silt-loam soil was collected at SAREC. A sample of this soil was sent to the University of Arkansas Diagnostics Laboratory in Fayetteville, Arkansas for analysis of chemical and physical properties (Table 1). Soil was dried in the greenhouse at 35° C for 2 weeks and 4.5 kg was added to 1-gallon buckets (base diameter - 19.05 cm, height - 18.7 cm, top diameter - 20.32 cm). Soil moisture were calculated using the formula used in Study B.1

The experiment was a split-plot randomized complete block design with four replications with herbicide as main plot and Atonik concentration as split plot. The experiment consisted of 28 treatments including non-treated checks. FullpageTM hybrid 'RT7321FP' rice was used. Seed lots (400 g each) were treated with seven concentrations of Atonik (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ml kg⁻¹ seed). After treatment, seeds were incubated in Ziplock bags for 2 hr, and air-dried in a dishpan overnight at room temperature. The rice seeds were planted in 14 seed buckets⁻¹ at 100% field capacity at the appropriate depth. The herbicides were applied immediately after planting, in a spray chamber fitted with a motorized boom with two flat-fan 1100067 nozzles (TeeJet, Glendale Heights, IL) calibrated to deliver 187 L ha⁻¹. The herbicide treatments, trade names, application rate, application timings, and manufacturers are listed in Table 3.

After herbicide application, the buckets were moved to a greenhouse and maintained at $32/25 \text{ °C} \pm 3 \text{ °C}$ day/night temperature with a 16-hour photoperiod. The buckets were watered to 100% field capacity daily and maintained at this moisture level. Ten days after planting, seedlings were counted and thinned to 4 bucket⁻¹. The buckets were flooded (2.54 cm above the soil surface), and urea (168 kg ha⁻¹) was applied in three splits (30, 60 and 90 days after planting). Potassium at 56 kg ha⁻¹at before planting. This experiment was conducted twice.

Data collection and analysis

Emerged seedlings were counted at 1 WAT. Rice injury was evaluated visually at 2, 4, and 6 WAT. At 6 WAT, plant height was also measured and 3 plants bucket⁻¹ were cut at the soil surface, dried at 60 C for 4 to 5 days, and weighed. The remaining plant was cultured until maturity and grain yield per plant was recorded.

In this study, crop injury data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC), and means were separated using Tukey's protected least significant difference (LSD) $\alpha = 0.05$. Plant height and biomass data were analyzed using the fit-model platform, JMP Pro 16.1(SAS Institute Inc., Cary, NC). In the analysis, herbicide, Atonik seed treatment, and their interactions were analyzed as fixed effects. Block, block x herbicide, and block x Atonik, were included in the model as random effects. All data were subjected to analysis of variance (ANOVA), and treatment means were separated by Tukey Kramer LSD test at $\alpha = 0.05$. All quantitative data were reported as actual values. Crop injury was evaluated relative to the non-treated controls.

Study C: Atonik seed treatment with delayed pre-emergence herbicides

Study C.2 and C.3. Response of rice to Atonik seed treatment and delayed pre-emergence herbicides in clay and silt loam soil in the field

Field trials were conducted at the Rohwer Research and Extension Center (37°12' 49.272" S, 142° 34' 4.758" E) Rohwer, Arkansas in the summer of 2021 in silt loam (block W1C) and clay loam soil (block BW6A). Soil samples were collected from the experimental site and sent to the Agricultural Diagnostic Laboratory at the University of Arkansas, Fayetteville, AR for analysis (Table 1).

The experimental design was a split-plot randomized complete block with 4 replications with herbicide as main plot (4 levels) and Atonik concentration as a subplot (5 levels). Details about the herbicide treatments are listed in Table 3.

The experimental site was tilled conventionally and 1 week before planting (WBP) the whole experimental area was over-sprayed with paraquat and glyphosate at the rate of 1.276 and 1.348 kg ai ha⁻¹. The soil test did not warrant preplant application of nitrogen. The urea was applied

in two splits using an airplane: first application of 123 kg ha⁻¹ was made at 4 WAP, and a second application of 335 kg ha⁻¹ was made at 8 WAP.

'RT7321FP' was planted using an Arca seed drill on May 23, 2021, at two locations with a base seed treatment of Atonik concentrations at 0, 0.375, 0.750, 1.50 and 3.0 ml kg⁻¹ seed, respectively. Plots were 1.5 m wide X 4.8 m long (including 9 rows of rice and 19.05 cm of spacing). The herbicides were applied 2 d after planting (delayed pre-emergence) using a CO₂pressurized backpack sprayer attached to a hand-held spray boom with three 8002 flat fan nozzles (Teejet, Glendale Heights, IL) spaced 45.72 cm apart calibrated to deliver 187 L ha⁻¹ at 206 kPa⁻¹. Before planting, the test area was treated with paraquat and glyphosate at the rate of 1.276 and 1.348 kg ai ha⁻¹ to control barnyardgrass and broadleaf. Following this application, the other maintenance herbicides were applied at two-leaf stages (Appendix Table 2)

Data collection and analysis:

Rice seedlings were counted at 2 WAT from 0.5-m length of a row at two locations per plot. Rice injury was visually evaluated relative to the nontreated control 2, 4, and 6 WAT +/- 2 days. The number of tillers m⁻¹ and panicles m⁻¹ were counted at maturity. Rice was harvested using a small plot combine that records yield per plot and grain moisture. Rice grain yield was expressed on a kg ha⁻¹ basis and adjusted to 12% moisture.

In this study, crop injury data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC), and means were separated using Tukey's protected least significant difference (LSD) $\alpha = 0.05$. In contrast, plant height, panicle count, and yield were analyzed using the fit-model platform, JMP Pro 16.1(SAS Institute Inc., Cary, NC). In the analysis, herbicide, Atonik seed treatment, and their interactions were analyzed as fixed effects. The block, block x herbicide, and block x Atonik were included in the model as random effects. All data were

subjected to analysis of variance (ANOVA), and treatment means were separated by Fischer's protected LSD test at $\alpha = 0.05$. All quantitative data were reported as actual values. Crop injury was evaluated relative to the non-treated controls.

Results

Study A: Atonik seed treatment with preplant herbicides

Study A.1. Response of rice to Atonik seed treatment and preplant herbicides in the silt loam soil in the field

The Atonik and herbicide treatments did not affect crop stand (Appendix Table 5). Rice injury was not assessed at 2 WAT because of flooding (534.16 mm rainfall) (Appendix Table 3). At 4 WAT, the main effects and the interaction of herbicide and seed treatment were significant on rice injury (Table 4). Injury symptoms were chlorosis, necrosis, and stunting. All plots with Atonik seed treatment had at least 10% injury regardless of herbicides applied. Without seed treatment, Trivence[®] (Canopy+ flumioxazin) and Canopy[®] (metribuzin + chlorimuron) caused the highest injury at 70% and 65%, respectively. With Atonik seed treatment at 0.225 % v/wt. Rice injury was reduced to 61% from 65% for Canopy herbicide. Only Valor treatment had less than 5% injury. By 6 WAT, the rice plants generally recovered; however, the injury from the Trivence and Canopy herbicide treatments remained high (Table 5). Seed treatment with Atonik significantly reduced injury from 2,4-D herbicide at 6 WAT. The injury from all other herbicides except Canopy and Trivence was < 30%.

The interaction effect between the two factors on plant height and number of tillers m⁻¹ was not significant, but the main effects were significant. The plants treated with Canopy and Trivence were shorter. The height differences between the Atonik seed treatments were expected because previous research has shown that rice with Atonik seed treatment were taller than rice without seed treatment [28]. The highest number of tillers (294 m⁻¹) was produced with Grandstand + Stinger treatment and the least with Trivence (119 number m⁻¹). Atonik seed treatment did not increase the tiller number, averaged across herbicides (Table 5).

There was no significant herbicide x Atonik seed treatment interaction effect panicle count in both soil types. In silt loam soil, rice seeds treated with Atonik at 0.225 % v/wt produced the highest number of panicles m⁻¹, significantly greater than of the non-treated. The highest number of panicles (45 m⁻¹) was recorded in plots treated with 0.225% v/wt of Atonik. The increase in panicle number with Atonik seed treatment resulted in increased yield with a few preplant herbicides.

The main effects of Atonik seed treatment and herbicide and their interaction on yield were not significant (Table 4). The Atonik seed treatment numerically increased rice yield with all the herbicides. The highest yield (12838 kg ha⁻¹) was recorded from plots treated with Valor + Atonik while the minimum yield was obtained from plots with Trivence (9773 kg ha⁻¹). Thus, there was an observable yield advantage from Atonik seed treatment, but this was generally low.

Study A.2. Response of rice to Atonik seed treatment and preplant herbicide in the clay loam soil

The Atonik and herbicide treatments did not affect crop stand (Appendix Table 5). In clay loam soil, the interaction between herbicide x Atonik seed treatment on all response variables evaluated was not significant (p > 0.5). The main effect of herbicide was significant for crop injury, tiller, panicle count, and yield (Table 4). Rice emerged about one week from planting and injury symptoms were visible by 2 WAT (data not shown). At 4 WAT, herbicides belonging to MOA group 7 (diuron, linuron, and propanil) and group 14 (flumioxazin and saflufenacil) caused < 10% injury (Table 6). Trivence, and Canopy injured rice 80% and 88% respectively. Injury from the tank mix treatments including triclopyr + clopyralid, 2,4 - D + thiobencarb, and fluridone + diuron were negligible to none (0 to 6%). The relatively new group 14 rice herbicide (saflufenacil) caused only 6% injury at 4 WAT. Saflufenacil was considered a standard treatment to which all other herbicide treatments could be compared. Our hypothesis was Atonik seed treatment with rice will reduce the herbicide stress and improve the rice yield.

At 6 WAT, rice plants treated with Atonik began to recover from herbicide injury while those without Atonik seed treatment continued to worsen. With some herbicides, Atonik-seed treatment reduced rice injury when compared to the standard treatment saflufenacil. The most noticeable benefit was observed with flumioxazin which caused 13% injury without seed treatment and < 6% injury with Atonik seed treatment. Overall, the Atonik seed treatment provided some safening from damage manifested in better recovery from injury with time.

Rice crop stand at 4 WAT, in Atonik and herbicide-treated plot were comparable to the saflufenacil treatment. Rice densities in plots treated with flumioxazin, linuron, and diuron were comparable to saflufenacil treatment. Plots treated with Canopy and Trivence had low rice densities (72 and 77 seedlings m⁻¹ respectively) compared to the standard treatment (97 seedlings m⁻¹) (Table 6).

The interaction effect between seed treatment and herbicide on plant height was not significant, but the herbicide main effect was significant (Table 3). Rice was significantly stunted in plots treated with Trivence and Canopy regardless of Atonik seed treatment (11 and 14 cm, respectively) compared to plants in the saflufenacil treatment (30 cm).

The interaction effect of seed treatment and herbicide on yield was significant (Table 6). The main effect of herbicide was also significant, but Atonik seed treatment was not. Rice treated with oxyfluorfen and with Atonik seed treatment produced the highest yield of 12044 kg ha⁻¹ (Table 6). Without seed treatment, rice treated with linuron produced numerically less (9780 kg ha⁻¹) when compared to rice with Atonik seed treatment and linuron (10103 kg ha⁻¹). However, this pattern was not true with other herbicides. For example, rice treated with Canopy herbicide, without Atonik seed treatment, produced numerically higher yield (6649 kg ha⁻¹) than when rice was treated with same herbicide and without Atonik seed treatment. Of all the herbicide treatments, 2,4 - D + thiobencarb produced the highest rough rice yield of 11532 kg ha⁻¹, averaged across Atonik rates. The average RRVP (Rice Research Verification Program) yield of 'RT7321FP' in 2021 was 11,499.80 kg ha⁻¹. This indicates that Atonik seed treatment afforded only minimal yield increases for this rice variety.

Study B: Atonik seed treatment and pre-emergence herbicides

Study B.1. Response of rice to Atonik and the pre-emergence herbicides in the greenhouse

Overall, the interaction between the herbicide and Atonik seed treatment was significant on rice injury at all evaluation times in the first run of the experiment (Table 8). However, there was no interaction effect in the second run. Cold temperature in the greenhouse during the second run (< 10 °C) had generally suppressed the growth of rice (Table 9). Except for quinclorac + thiobencarb, all other treatments caused less than 15% injury to rice. However, the quinclorac + thiobencarb treatment caused more than 20% injury on average across Atonik concentrations. This was observed at all the injury evaluations and was consistent across both runs of the experiment (Table 8 and 9). These results were consistent with the field experiments as well. The quinclorac + thiobencarb herbicide combination has caused the highest injury to rice when seeds are treated with 2.5 and 3.0 ml of Atonik. This was consistent across runs and all injury evaluations. The levels of injury associated with pre-emergence herbicides are consistent across two runs of this experiment, suggesting that quinclorac + thiobencarb causes a high risk for injury to 'RT7321FP'. However, the Atonik seed treatment at 2 ml kg⁻¹ seed resulted in the lowest injury of < 5% in two runs, across herbicides. At 6 WAT, the rice injury was >15% with quinclorac + thiobencarb.

The two-way interaction between Atonik seed treatment and herbicide effect was significant on biomass (Table 7). The main effect of Atonik seed treatment was significant only in the second run. The rice biomass was greater in the second run because plants were placed outdoors after evaluating injury % at 4 WAT (Table 7). The highest biomass of 14 g 3 plants⁻¹ was produced when quinclorac + pendimethalin was applied to rice treated with 0.5 ml of Atonik kg⁻¹ seed. In contrast the lowest biomass (8 g 3 plants⁻¹) was produced when quinclorac + thiobencarb was applied to rice treated with 1.5 ml of Atonik kg⁻¹ seed. These results were observed only in the 2nd run of the greenhouse experiment (Table 8). Overall, biomass produced from the herbicide treatments + Atonik seed treatment were greater than rice without seed treatment and without herbicide.

Study B: Atonik seed treatment with pre-emergence herbicides.

Study B.2. Response of rice to Atonik seed treatment and pre-emergence herbicides in the silt loam soil in the field

The Atonik and herbicide treatments did not affect crop stand (Appendix Table 6). At 4 WAT, there was no interaction effect on rice injury and had significant effect of herbicide on injury (Table 10). Injury to rice was < 50% with all the pre-emergence herbicides except quinclorac + pendimethalin (> 60% injury) irrespective of Atonik seed treatment (Table 11). Seed treatment with Atonik at 3.0 ml Atonik kg⁻¹ of seed was significantly superior and caused less injury (<40%) to rice when compared to other Atonik rates, averages across the herbicides. At 6 WAT, the response pattern was similar with respect to rice injury. However, the interaction between the two factors and main effects were significant on tillers. The highest number of tillers (442 m⁻¹) were

produced when quinclorac + pendimethalin was applied to rice that had been treated with 0.35 ml Atonik kg⁻¹ of seed. Rice treated with the same Atonik rate, and treated with quinclorac + clomazone, produced only 219 tillers m⁻¹. The herbicide did not significantly affect plant height or panicle count. The Atonik seed treatment had little effect on the rice yield. Overall, the yield ranged between 11156 and 13709 kg ha⁻¹.

Study B: Atonik seed treatment with pre-emergence herbicides

Study B.3. Response of rice to Atonik seed treatment and pre-emergence herbicides in the clay loam soil in the field

The Atonik and herbicide treatments did not affect crop stand (Appendix Table 6). The interaction effect between seed treatment and herbicide was not significant on any of the response variables. The Atonik seed treatment effect was not significant. However, there was an herbicide effect on rice injury at 4 WAT, tillers, and panicle count. (Table 10). Generally, crop injury less than 10% is acceptable. At 4 WAT, all herbicide treatments, including no herbicide, resulted in more than 25% injury to rice, averaged across Atonik concentrations. In particular, quinclorac + pendimethalin caused the highest (45%) injury. While, seed treatment with only Atonik caused 27% injury to rice, the average of Atonik concentrations. The injury symptoms were stunting, chlorosis and loss in crop stand. On the other hand, among Atonik concentrations, seed treatment with the 3.0 ml kg⁻¹ of Atonik resulted in the highest injury (~ 40%) to rice regardless of the preemergence herbicide. The lowest injury (32%) observed when rice seeds were treated with 1.5 ml Atonik kg⁻¹ seed, irrespective of pre-emergence herbicide. Although rice had recovered to some extent by 2 weeks following this evaluation, injury remained high for all the herbicide treatments compared to rice without seed treatment and without pre-emergence herbicides. At 6 WAT, all the herbicide treatments had caused < 20% injury (Table 12). Analysis between the Atonik concentrations showed that two rates of Atonik 1.5, and 3.0 ml kg⁻¹ caused less injury in the clay loam soil, averaged across herbicides.

Similar to silt loam soil, there was no interaction effect between the two factors, however, the herbicide main effect was significant on tillers and panicle count (Table 10). The highest number of panicles was recorded from the plots that received quinclorac + clomazone (83 panicles m⁻¹), averaged across Atonik seed treatment rates. Rice yield was not influenced by at least one of the two factors and their interaction. Overall, the yield ranged between 10707 and 12438 kg ha⁻¹ (Table 12) which is numerically 5% lower than that from the silt loam soil. The average RRVP yield of 'RT7321FP' hybrid rice from clay loam soil is 11499.80 kg ha⁻¹.

Study C: Atonik seed treatment with delayed pre-emergence herbicides

Study C.1. Rice Response to Atonik seed treatment and delayed pre-emergence herbicides in the greenhouse

The two-way interaction between herbicide and Atonik is significant on the injury at 2, 4 WAT, and biomass; the herbicide was also significant on Injury at 2,4 and 6 WAT. The results were consistent across both runs of the experiment (Table 13).

At 2 and 4 WAT, except with quinclorac + thiobencarb treatment, all other treatment combinations caused less than 15% visible injury (Table 14). Quinclorac + thiobencarb caused numerically the highest injury of 18%, average across Atonik concentrations. With the highest concentration of Atonik (3.0 ml kg⁻¹ of seed), quinclorac + thiobencarb caused 20% and 15% injury, at 2 and 6 WAT, respectively. By 6 WAT, rice injury was generally < 5%, but the injury caused by quinclorac + thiobencarb was higher (10%) compared to other treatments. Overall, quinclorac + thiobencarb caused the highest injury to rice at all the evaluations. In the second run, the injury trend was similar to the first run of the greenhouse except for injury at 2 WAT. The

herbicide quinclorac + thiobencarb combinations caused 24% injury, averaged across Atonik concentrations (Table 15).

The two-way interaction effect was significant on biomass, across runs. However, the biomass produced in the second run was greater than the first run because plants in the second run were placed outdoors after collecting injury at 4 WAT evaluation (Table 15). The highest biomass of 13 g 2 plants⁻¹ was produced when quinclorac + clomazone was applied to rice treated with 2.0 ml kg⁻¹ of seed. The rice seeds were treated manually with Atonik by diluting the higher concentration to a lower concentration. In contrast, the lowest biomass was produced when quinclorac + thiobencarb was applied to rice treated with 1.5 ml kg⁻¹ of seed. Overall, when compared with the other treatments, quinclorac + thiobencarb resulted in the highest above-ground biomass despite causing the highest injury to rice in all the injury evaluations.

Field experiments conducted in silt loam and clay loam soil showed that the Atonik seed treatment had no significant effect on yield. Additionally, there was no interaction effect on any of the injury evaluations in both soil types (Table 10). In both soil types, the highest rice injury occured when quinclorac + pendimethalin were applied at 1x rate $(0.336 + 1.12 \text{ kg ai } \text{ha}^{-1})$ respectively (Table 11).

Study C: Atonik Seed treatment with delayed-pre-emergence herbicides.

Study C.2 Response of rice to Atonik seed treatment and delayed pre-emergence herbicides in silt loam soil in the soil

The Atonik and herbicide treatments did not affect crop stand (Appendix Table 6). The two-way interaction was not significant (P > 0.05) between herbicide and Atonik seed treatment for all the response variables except for tiller count. However, the main plot factor and subplot factor were significant on tillers only (Table 16). At 2 WAT, injury data was not evaluated

because the entire experimental site was flooded within 10 days after herbicide application (Appendix Table 3). The plants were submerged in water for almost seven days. At 4 WAT, there was no interaction effect between herbicide and Atonik seed treatment and no main effects of herbicide and Atonik seed treatment on rice injury (Table 16). At 6 WAT, the main effect of Atonik seed treatment on rice injury was significant. The injury level increased gradually with increasing rate of Atonik. This showed that seed treatment with Atonik generally did not alleviate rice injury from soil-applied herbicides. Without Atonik seed treatment, the rice injury was less than 10%; however, with 3.0 ml kg⁻¹ of Atonik, rice injury was 20% (Table 16). Overall, quinclorac + pendimethalin caused the highest injury at both the injury evaluation times in the silt loam soil, averaged across Atonik seed treatment rates.

Plant height, number of panicles, and rice grain yield were not influenced by either of the factors (Table 16). The two-way interaction was not significant (P > 0.05) between herbicide and Atonik seed treatment for all the response variables. The effect of Atonik seed treatment on rice yield was not significant, but numerical improvements were detected. Rice grain yield ranged from 11161 kg ha⁻¹ to 14245 kg ha⁻¹ (Table 17). The lowest yield of 11161 kg ha⁻¹ was recorded from the plots treated with 0.75 ml Atonik kg⁻¹ rice seed and no herbicide. The highest yield of 14245 kg ha⁻¹ was recorded from plots treated with quinclorac + pendimethalin and 0.375 ml Atonik kg⁻¹ rice seed.

Study C: Atonik seed treatment with delayed pre-mergence herbicides

Study C.3. Response of rice to Atonik seed treatment and delayed pre-emergence herbicide in clay loam soil in the soil

The main effect of herbicide was significant on rice injury at 4 WAT, Tillers, and panicle count; and there was no interaction effect between the two factors for any of the response variables

(Table 16). The two-way interaction between herbicide and Atonik seed treatment on rice injury was not significant, but the main effect of herbicide was significant. At 2 WAT, injury symptoms were noticeable in some plots, but injury was not scored due to flooding (Appendix Table 4) we did not collect the injury at 2 WAT. At 4 WAT, all the herbicide treatments caused more than 20% injury, irrespective of Atonik concentrations (Table 18). The quinclorac + pendimethalin treatment caused the highest injury across all Atonik concentrations compared to other herbicide treatments. With the highest concentration of Atonik (3 ml kg⁻¹ seed) quinclorac + pendimethalin caused 50% injury to rice. At 6 WAT, rice injury was generally 20%, except quinclorac + thiobencarb caused (23%) injury to rice, across Atonik concentrations.

The main effect of herbicide was significant on tillers and panicle count. However, there were no interaction effect or any other main effect on grain yield (Table 16). The highest number (345 m^{-1}) of tillers were produced with quinclorac + thiobencarb herbicides were applied compared to other herbicide treatments, averaged across Atonik rates. However, the highest number of panicles (70 panicles m⁻¹) was produced from no herbicide treatment, averaged across Atonik concentrations. Across all treatments, the rice grain yield ranged from 12168 kg ha⁻¹ to 16306 kg ha⁻¹. The highest yield of 16306 kg ha⁻¹ was obtained from plots treated with quinclorac + thiobencarb and 0.75 ml Atonik kg⁻¹ rice seed. The lowest yield of 12168 kg ha⁻¹was recorded from plots treated without herbicide with 3.0 ml Atonik kg⁻¹ rice seed.

Discussion

Study A: Atonik seed treatment with preplant herbicides

Study A.1. and A.2. Response of rice to Atonik seed treatment and preplant herbicides in the field

The analysis of this study demonstrated that Atonik seed treatment with pre-emergence herbicide affects the emergence, growth and as well as yield. All these factors are dependent on the concentration of Atonik, mode of action of pre-emergence herbicide and the rate of herbicide. Previous literature reveals that seed treatment with Atonik reduced the abiotic and biotic stresses, helping the plant to thrive throughout the growing season [29]. Therefore, this research was conducted to examine if Atonik seed treatment could improve rice performance with soil-applied herbicides (preplant, pre-emergence, and delayed pre-emergence).

A residual herbicide is defined as an "herbicide that persists in the soil and injures or kills germinating weed seedlings for a relatively short period of time after application [30]. Herbicide adsorption and transport in soil are important factors that determine herbicide efficacy and residual activity. The adsorption of herbicide molecules to soil is affected by the chemical and physical properties of the herbicide, soil texture, the type of clay mineral, moisture, and the organic matter present in the soil [31]. Likewise, rate of application, mobility of herbicide; rainfall, topography, and climate play an important role in the herbicide efficacy [32,33]. In Arkansas, herbicides that are applied preplant in rice production includes thiobencarb, clomazone, quinclorac, pendimethalin, imazethapyr, and imazosulfuron [34]. The advantage of residual herbicide application at planting is increasing flexibility with timings of post-emergence herbicide applications and helping achieve yield potential [20,35–39].

To date, little attention has been paid to the use of biostimulants to increase crop tolerance to herbicides. For this reason, a study exploring the use of Atonik with preemergence and delayed preemergence experiments was conducted. Also, examine the effects of Atonik, a nitro-phenolatebased biostimulant, on rice grown in the field and under greenhouse conditions.

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Rice injury was higher in clay loam soil than in silt loam soil. Among all the herbicides, the increase in rice injury in clay soil was expected since chlorimuron herbicide activity is highly dependent on soil pH (pH > 7) [40]. Chlorimuron volatility is directly proportional to soil pH, and adsorption to soil particles is inversely proportional to moisture levels [41]. The rice injury from chlorimuron in clay loam soil is 25% higher than silt loam soil. Similarly, rice injury from Canopy and Trivence treatment was higher in clay loam soil than in silt loam soil. Overall, plots treated with Trivence or Canopy herbicides caused the highest injury at both injury evaluations. Trivence can be applied 14 days prior to soybean planting to control broadleaf weed species and to avoid crop injury [42]. This herbicide is not labeled for rice, but was tested here to determine if seed treatment with Atonik can safen it for use in rice preplant as in soybean. Among the herbicide treatments, only the 2,4-D has benefited from the Atonik seed treatment, which reduced the rice injury from 45 to 27%. Despite its being labeled for rice, 2,4-D can cause injury depending on the timing of application [43]. Generally, injury caused from 2,4-D can be manifested as leaf rolling, failure of panicles to emerge, a reduction in kernels per head, and a delay in maturity [44]. On the other hand, all the herbicide treatments caused less than 15% crop injury including saflufenacil. Saflufenacil is a broad-spectrum, selective herbicide used in cereals small grains, corn, sorghum, soybeans, and sunflowers, chickpeas, cotton, for the control of broadleaf weeds [45,46]. In rice, saflufenacil has been reported to cause < 5% injury [45-48]. Such low level of injury does not affect yield.

Contrary to expectation, Atonik seed treatment reduced rice height and tillers 6 WAT in all herbicide treatments including the standard herbicide (saflufenacil). In contrast, many studies showed that seed treatment with Atonik has increased the plant height from 4 to 16 cm in cotton and 5 to 9 cm in tomato [12,49,50]. The number of tillers is most closely related to panicle as well

as rice grain production [51]. In this study, except for fluridone herbicide treatment with Atonik, all other treatments resulted in fewer tillers and panicles. This response is in line with the fact that Atonik seed treatment did not result in healthier rice seedlings with or without herbicides.

Rice yield response to Atonik seed treatment and preplant herbicides

Considering the lack of positive response to Atonik seed treatment in vegetative growth, all herbicide treatments generally benefited in small amounts from Atonik seed treatment with respect to yield. This was observed only in silt loam soil. Although the yield increase was not significant, rice yield numerically improved between 4 and 10% compared to the non-treated check. It seemed that the Atonik seed treatment had some residual benefit on carbohydrate metabolism, which resulted in slight yield increases despite the lack of benefit on tillering or panicle counts. A similar response to Atonik seed treatment was reported by Elankavi et al. (2009), on rice [52]. Increase in rice yield due to the activity of Atonik on reproductive development rather than vegetative development. Similarly, rice yield increased with the Atonik seed treatment only in the silt loam soil, regardless of the use of herbicides. Among the herbicide treatments, the highest yield of 12838 kg ha⁻¹ was produced from the flumioxazin + Atonik seed treatment. Without seed treatment rice has produced 11095 kg ha⁻¹, which is 13% yield reduction without Atonik seed treatment. Out of 11 herbicides applied to clay loam soils, only three follow this pattern: linuron, oxyfluorfen, and triclopyr +clopyralid treatments had benefited from Atonik seed treatment. The yield increase with Atonik seed treatment may be due to an increase in the number of productive tillers (15, 6 & 3 %), or the number of panicles (0, 11 & 10%), which are closely associated with seed yield resulting in slightly improved productivity (4,19, and 8%) [52]. An Atonik seed treatment experiment was conducted in Ghana using five levels of Atonik ranging from 40 to 600 ml ha⁻¹ with the mean difference of 50 ml ha⁻¹. This experiment was conducted using two different rice varieties (Agra Rice and Jasmine 85)

They found that Atonik (500 ml ha⁻¹ improved the root growth of rice. As a result, it improved uptake of phosphorous and potassium and increased accumulation of these nutrients in the rice stem, leaves, and grain [50]. In the current study, the Atonik seed treatment with herbicide had a significant influence on rice yield in clay loam soil. Overall, four out of twelve herbicide treatments benefited from Atonik seed treatment in improving rice yield.

Study B: Atonik seed treatment and pre-emergence herbicides

Study B.1. Response of rice to Atonik and the pre-emergence and delayed pre-emergence herbicides

Research has shown that biostimulants can also have a positive influence on plant growth and nutrient assimilation and improve their tolerance to abiotic stresses [53,54]. For this reason, a study on the optimization of Atonik with pre-emergence and delayed pre-emergence experiments was carried out. Additionally, examine the effect of nitrophenolates-based biostimulant, Atonik, in rice crop grown under field and controlled conditions in a greenhouse.

In this current research, in the field, rice injury from herbicide treatments differed between silt loam and clay loam soil. In general, injury from pre-emergence or delayed pre-emergence herbicides was higher in silt loam soil than clay loam soil. The variation in rice injury across soil types is likely due to different herbicide behavior in these two soil environments [50,55,56]. Clay particles are negatively charged and have a large surface area. As a result, soils high in clay content have higher capacity to adsorb or tie up herbicides and require higher herbicide rates to kill weeds than silt loam soil. This was true for both pre-emergence and delayed pre-emergence experiments. Considering that the same herbicide rate was applied to both soils, it is logical that herbicide

activity would be lesser in clay than in silt loam soil. On a grand scales, the persistence of herbicides is influenced by herbicide rate and chemical prosperities, soil type, soil temperature, soil moisture, and cultivation practice [57]. Among these factors herbicide rate, soil type, and soil moisture play a significant role in herbicide selectivity on rice. Street and Landham, 1996 [58] reported that when pendimethalin was applied 1, 4, or 7 days after planting, rice injury ranged from 0 to 30%.

Although the herbicides and Atonik concentrations were the same in both experiment locations; rice injury varies from one soil type to the other (silt loam and clay loam soil) because of different organic matter, clay content, soil moisture, and CEC content (Table 1). Clomazone, thiobencarb, pendimethalin, imazethapyr, and thiobencarb are the only delayed preemergence herbicide options in US rice production [7]. Quinclorac was commonly used in all herbicide treatments because it provides excellent residual control of barnyardgrass, broadleaf signalgrass, morningglory, hemp sesbania, and northern jointvetch (*Aeschynomene virginica*) [59].

In the greenhouse at 2 WAT, the application of quinclorac + thiobencarb and quinclorac + clomazone caused the highest injury (> 20%) regardless of application timing when compared to other herbicide treatments. However, in the field, quinclorac + pendimethalin caused the highest injury (> 40%) compared to other herbicide treatments, regardless of application timing. York et al [60] reported that clomazone applied immediately after planting at 0.336 kg ai ha⁻¹ on silt loam or clay loam soil caused up to 25% injury on rice at the seedling stage, but this had no effect on yield. In the field at 4 and 6 WAT, the rice injury was generally less than < 20% irrespective of herbicide application timings and Atonik concentrations, averaged across herbicides. Overall, Atonik seed treatment did not significantly reduce the stress with the pre-emergence or delayed

[61] and quinclorac < 8 % injury to rice relative to delayed pre-emergence application [62].

Atonik treated plants increased plant height numerically, but not statistically significant. We observed a similar trend in our experiment as well. Dunand, 1998 [63] also reported that the application of plant growth regulators significantly increases plant height in rice. The reason for the increase in plant height may be due to increased cell division, cell enlargement, and cell elongation [64]. Also, Adam et al.,(2011) reported that significant increase in the number of tillers produced. Seed treatment with Atonik resulted in a 35% more tillers than non-treated. Likewise, 0.75, 3.0, and 1.5 ml Atonik ha⁻¹ resulted in 31%, 29%, and 15% higher productive tillers, respectively, than rice without Atonik. The number of productive tillers is directly proportional to the number of panicles produced per plant [65]. Additionally, hybrid rice relies mainly on tillers to obtain a desirable population and about [52].

It was observed in only the second run of the greenhouse that rice shoot biomass increased with increasing Atonik seed treatment concentrations. Due to a) better uptake and accumulation of mineral nutrients [66] and b) an increase in plant height in Atonik seed-treated plants [55]. As a result, high level of biomass production.

In clay loam soil, Atonik-treated plants ehcbitis more reproductive development rather than vegetative [66]. In both the pre-emergence field studies, some of the Atonik seed treatment concentrations resulted in numerical (0 to 900 kg ha⁻¹) increases in grain yield relative to the non-treated check. Small yield increases ranged from 1.9 to 5.2%. In the present study, enhanced rice tolerance to pre-emergence herbicide stress was observed from the seed treatment with Atonik. As a result, rice grain yield increased by 15% when rice seeds were treated with 0.375 ml Atonik kg⁻¹ rice seed, and quinclorac plus thiobencarb herbicide was applied in clay loam soil relative to no

herbicide and no Atonik seed treatment. A similar response was observed in silt loam soil, but the highest yield was produced with Atonik rate of 1.0 ml kg⁻¹ of seeds without herbicide. Overall, the beneficial concentration of Atonik ranged between 0.75 to 3.0 ml kg⁻¹ seed. In the pre-emergence experiment, the yield ranged from 10707 kg ha⁻¹ to 12960 kg ha⁻¹while in the delayed preemergence experiment yield was 11161 kg ha⁻¹. to 14791 kg ha⁻¹. Kalavathi et al., [67] and Shihua et al., [68] reported that hybrid rice (TNRH2) treated with GA3 as a seed treatment in India and chine. Also they reported that rice yield significantly increased with the application of PGR and bigger panicle associated with higher number of grains per panicles which results in high productivity [67,68]. It is possible that the increase in grain yield has been caused by the activity of Atonik on rice roots, which has led to an increase in potassium accumulation in rice stems, leaves, and grains [69]. Atonik has a positive effect on crop growth and development by improving tolerance to adverse environmental conditions, such as salinity, drought, and temperature [16].
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Table 1. Soil chemical properties at the sites where the experiments were conducted in 2021 and 2022.																
Location	Soil type	\mathbf{P}^{H}	Р	Κ	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	В	Sand	Silt	Clay
								mg	kg ⁻¹						(%))
SEREC ^a , Rohwer	Silt	7	84	186	1326	272	6	6	235	106	4	2	0	26.9	54	18.9
SEREC ^a , Rohwer	Clay	7	41	212	2944	636	16	5	314	44	3	2	1	0.4	46	53.6
SAREC ^b , Fayetteville	Silt	7	49	103	1073	40	7	7	88	213	2	1	0	27.1	59	13.9

 Tables

 Table 1. Soil chemical properties at the sites where the experiments were conducted in 2021 and 2022

^aAbbreviations: SEREC – Southeast Research and Extension Center, Rohwer, AR

^bAbbreviations: SAREC – Milo L. Shult Agricultural Research and Extension Center, Fayetteville, AR

Soil texture was analyzed using the hydrometer method.

Herbicide treatments	Trade Name	Application rate (kg ai ha ⁻¹)	Manufacturer
2,4 - D	Weedar 64	1.12	Bayer CropScience, Research Triangle, North
			Carolina, USA
2,4-D + thiobencarb	Weedar 64 +	1.12 + 4.483	Bayer CropScience, Research Triangle, North
	Bolero		Carolina, USA; Valent, USA, Corporation,
			walnut Creek, CA
chlorimuron + flumioxazin +	Trivence	0.07 + 0.02 + 0.24	Corteva, Agri Sciences, Indianapolis, IN
metribuzin			
diuron	Direx	0.56	ADAMA, 3120 Highwoods Blvd, Raleigh, NC
flumioxazin	Valor	0.062	Valent, USA, Corporation, walnut Creek, CA
fluridone	Brake	0.336	SePRO, Corporation, North Meridian, St.
			Carmel, NC
fluridone + diuron	Brake + Direx	0.336 + 0.56	SePRO, Corporation, North Meridian, Street
			Carmel, NC; ADAMA, 3120 Highwoods Blvd,
			Raleigh, NC
linuron	Linex	0.84	Novasource, 44th street suite, Phoenix, AZ
metribuzin + chlorimuron	Canopy	0.28 + 0.043	Corteva Agri Sciences, Indianapolis, IN
oxyfluorfen	Goal	0.28	Corteva Agri Sciences, Indianapolis, IN
saflufenacil	Sharpen	0.049	BASF, Corporation 26 Davis Drive, Research
	-		Triangle Park, NC
triclopyr + clopyralid	GrandStand +	0.28 + 0.28	Dow Agri Sciences, Zionsville Road,
	Stinger		Indianapolis, IN; Corteva Agri Sciences,
	<u> </u>		Indianapolis, IN

Table 2. Preplant herbicides tested on rice with and without Atonik seed treatment, Rohwer, AR, 2021.

^aAbbreviations: PPL- preplant herbicide application; 30 days before planting rice.

Table 3. Rice herbicides applied pre-emergence and delayed pre-emergence rice with different Atonik seed treatments, in Rohwer, and in the greenhouse at Fayetteville, AR, 2021 and 2022.

Herbicide Program	Application rate (kg ai ha ⁻¹)	Application timings	Manufacturer
quinclorac + thiobencarb	0.336 + 0.336	pre-emergence ^a ,	BASF Corporation 26 Davis Drive, Research
		delayed pre-	Triangle Park, NC; FMC corporation 2929 Walnut
		emergence ^b	Street Philadelphia, PA
quinclorac + clomazone	0.336 + 1.12	pre-emergence,	BASF Corporation 26 Davis Drive, Research
		delayed pre-	Triangle Park, NC; MC corporation 2929 Walnut
		emergence	Street Philadelphia, PA
quinclorac + pendimethalin	0.336 + 4.48	Pre-emergence,	BASF Corporation 26 Davis Drive, Research
		delayed pre-	Triangle Park, NC; Valent, USA, Corporation,
		emergence	Walnut Creek, CA

^aAbbreviations: pre-emergence application; immediately after planting the rice

^bdelayed pre-emergence application; two days after planting

Plant Tillers Panicle Yield Soil type Source of Variations Injury, 4 WAT^a Injury, 6 height (cm) (count m^{-1}) (count m^{-1}) (kg ha^{-1}) WAT Herbicide <.0001*b <.0001* <.0001* <.0001* 0.0003* <.0001* Clay loam Atonik rate 0.6951 0.0151 0.9925 0.2867 0.9126 0.2849 Herbicide x Atonik rate 0.6944 0.7105 0.0989 0.4637 0.1034 0.0448* Herbicide <.0001* <.0001* <.0001* 0.0021* 0.1713 0.2792 <.0001* <.0001* 0.0199* 0.0674 Silt loam Atonik rate 0.0160* 0.2413 Herbicide x Atonik rate 0.057 0.3458 0.4376 0.0315* 0.7296 0.2016

Table 4. ANOVA table listing P-values for rice response to some preplant rice herbicides with two doses of Atonik in Rohwer, Southeast Research and Extension Center, Rohwer, AR, 2021.

^bP-values were generated using a beta distribution in SAS and generalized linear mixed model in JMP Pro 16.1

*Abbreviations: significant factor effect at P(α =0.05)

Herbicide ^a	Atonik	In	jury, 4	Injury, 6	Plant height	Tillers	Panicle	Yield
		W	AT	WAT	(cm)	$(\text{count } \text{m}^{-1})$	$(\text{count } \text{m}^{-1})$	(kg ha^{-1})
2,4 - D + thiobencarb	0	2	EF ^b	6	31	284	46	11179
	0.225	5	EF	8	31	278	46	12265
2,4-D	0	46	BDAC	34	30	278	43	10886
	0.225	27	EBDAC	24	28	273	43	12768
chlorimuron + flumioxazin +	0	69	BA	69	21	119	44	9130
metribuzin	0.225	74	А	75	19	134	36	9773
diuron	0	5	EF	10	33	227	33	10766
	0.225	18	EBDACF	15	32	247	41	12346
flumioxazin	0	5	EDF	6	31	320	44	11095
	0.225	11	EDCF	12	32	272	40	12838
fluridone	0	4	EF	1	32	258	38	10510
	0.225	10	EDF	6	32	299	42	11854
fluridone + diuron	0	1	F	3	31	268	39	10791
	0.225	16	EBDCF	8	31	274	43	11834
linuron	0	2	EF	5	31	222	50	11893
	0.225	7	EDF	7	29	263	50	12095
metribuzin + chlorimuron	0	66	BA	63	20	139	46	9666
	0.225	64	BAC	69	19	124	46	11035
oxyfluorfen	0	3	EF	3	33	216	40	10879
-	0.225	10	EDF	10	32	206	52	12067
saflufenacil	0	3	EF	2	31	232	39	11046
	0.225	12	EDCF	12	31	206	51	12158
triclopyr + clopyralid	0	11	EDCF	13	32	299	40	11423
	0.225	29	EBDAC	19	30	289	49	12156
^c P-values			<.0001	0.117	0.4376	0.5263	0.6001	0.1357

Table 5. Effect of preplant herbicides on rice injury with and without Atonik seed treatment in silt loam soil, SEREC, Rohwer, AR, 2021.

^aAbbreviation: WAT: weeks after treatment; Herbicides were applied at 30 days before planting

^bMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP-values were generated using the SAS programming for injury; while JMP pro 16.1 used for plant height, panicle count and yield they were calculated using a generalized linear mixed model in JMP Pro 16.1

Herbicide ^a	Atonik	Injury, 4	Injury, 6	Plant height	Tillers	Panicle	Yield
		WAT	WAT	(cm)	(count m ⁻¹)	(count m ⁻¹)	$(kg ha^{-1})$
2,4 - D + thiobencarb	0	1	7	30	128	58	11811 A ^b
	0.225	8	8	29	134	62	11253 A
2,4-D	0	5	10	30	119	60	10611 A
	0.225	6	4	30	145	61	9911 A
chlorimuron + flumioxazin + metribuzin	0	88	81	11	60	38	10027 A
	0.225	90	86	9	60	29	9892 AB
diuron	0	4	8	30	150	54	11102 A
	0.225	2	10	29	173	53	9987 A
flumioxazin	0	1	13	31	150	65	10433 A
	0.225	7	6	30	40	60	9710 AB
fluridone	0	5	11	29	138	60	10578 A ^b
	0.225	7	8	30	157	70	9602 AB
fluridone + diuron	0	3	8	30	136	58	11346 A
	0.225	5	3	29	118	72	10940 A
linuron	0	4	9	29	132	51	9780 AB
	0.225	3	6	33	157	49	10103 A
metribuzin + chlorimuron	0	81	80	11	68	35	6649 BC
	0.225	79	75	14	78	35	5549 C
oxyfluorfen	0	6	10	26	124	48	9421 AB
	0.225	7	9	31	132	54	12044 A
saflufenacil	0	11	11	31	156	71	11090 A
	0.225	6	7	31	142	67	10928 A
triclopyr + clopyralid	0	7	9	30	136	57	9475 AB
	0.225	3	2	30	140	64	10344 A
^c P-values		0.4146	0.5981	0.0989	0.875	0.9126	0.045

Table 6. Effect of preplant herbicides on rice injury with and without Atonik seed treatment in clay loam soil, SEREC, Rohwer, AR, 2021.

^aAbbreviation: WAT: weeks after treatment; Herbicide were applied 30 days before planting.

^bMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP-values for injury were calculated using a beta distribution in SAS; while for tillers, panicle count, and yield they were calculated using a generalized linear mixed model in JMP

Sources of variation	Injury, 2 WAT ^a	Injury, 4 WAT	Injury, 6 WAT	Plant height (cm)	Biomass (g)
Herbicide	<.0001*	0.0003*	0.0011*	0.5404	0.0345
Atonik rate	0.0003*	0.0524	0.3066	0.0068*	0.2873
Herbicide x Atonik rate	0.0213*	0.0213*	0.0071*	0.0177*	0.0169*
Herbicide	<.0001*	<.0001*	0.0017*	0.0147*	0.1814
Atonik rate	0.1787	0.1021	0.2931	0.3184	0.0108*
Herbicide x Atonik rate	0.9258	0.2456	0.0801	0.9227	0.0214*
- -	Sources of variation Herbicide Atonik rate Herbicide x Atonik rate Herbicide Atonik rate Herbicide x Atonik rate	Sources of variationInjury, 2 WATaHerbicide<.0001*	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$

Table 7 Analysis of variance on the effect of primary pre-emergence rice herbicides and Atonik on rice injury, height, biomass, in the greenhouse, Milo J Shult Research and Extension Center, Fayetteville, AR, 2022

^bP- values for injury were calculated using a beta distribution in SAS; while for tillers, panicle count, and yield they were calculated using a generalized linear mixed model in JMP Pro 16.1.

*Abbreviations: significant factor effect at $P(\alpha=0.05)$

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Herbicide ^a	Atonik rate (ml kg ⁻¹ seed)	Injur WA	ry, 2 AT	Inju 4 W	iry, ⁄AT	Inju WA	ry,6 AT	I h	Plant eight (cm)	l (g	Biomass (of 2 plants ⁻¹)
No herbicide	0	-	-	-	-	-	-	56	AB ^b	8	ABC
	0.5	1	В	5	В	1	В	57	AB	8	ABCD
	1	1	В	1	В	3	В	57	AB	8	ABC
	1.5	1	В	1	В	1	В	61	А	7	ABCDE
	2	1	В	2	В	3	В	57	AB	8	ABC
	2.5	1	В	1	В	1	В	58	AB	9	А
	3	5	В	3	В	1	В	59	AB	9	AB
quinclorac +	0	14	В	14	В	8	В	56	AB	6	ABCDEFG
thiobencarb	0.5	9	В	8	В	7	В	56	AB	8	ABCDEF
(0.336 +	1	8	В	6	В	4	В	58	AB	3	G
0.336 kg ai	1.5	23	BA	21	BA	13	В	56	AB	6	BCDEFG
ha ⁻¹)	2	6	В	8	В	10	В	54	ABC	6	ABCDEFG
	2.5	29	BA	14	В	11	В	56	AB	4	DEFG
	3	48	А	46	А	49	А	45	С	7	ABCDEFG
quinclorac +	0	1	В	7	В	12	В	53	ABC	5	BCDEFG
clomazone	0.5	2	В	6	В	11	В	55	AB	6	ABCDEFG
(0.336 + 1.12)	1	4	В	3	В	5	В	55	AB	4	EFG
kg ai ha ⁻¹)	1.5	4	В	8	В	10	В	51	BC	4	CDEFG
	2	3	В	3	В	13	В	52	BC	6	ABCDEFG
	2.5	6	В	8	В	14	В	52	ABC	3	FG
	3	7	В	6	В	15	В	51	BC	5	BCDEFG
quinclorac +	0	1	В	8	В	8	В	54	AB	5	BCDEFG
pendimethalin	0.5	6	В	4	В	7	В	56	AB	7	ABCDEFG
(0.336 + 4.48)	1	2	В	2	В	7	В	57	AB	6	ABCDEFG
kg ai ha ⁻¹)	1.5	1	В	2	В	4	В	57	AB	6	ABCDEFG
	2	6	В	3	В	5	В	57	AB	5	BCDEFG
	2.5	14	В	10	В	11	В	55	AB	6	BCDEFG
	3	4	В	5	В	10	В	52	ABC	5	CDEFG
^c P-values		0.0	21	0.0	21	0.0	07	C	0.017		0.016

Table 8. Effect of primary pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury in the greenhouse, first run, Fayetteville, AR, 2021

^bPlant height was measured at 6 WAT (weeks after treatment), one plant per bucket⁻¹ ^cMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP- values for injury were calculated using a beta distribution in SAS; while for tillers, panicle count, and yield they were calculated using a generalized linear mixed model in JMP Pro 16.1.

	Atonik rate	Injury, 2	Injury.	Iniury.	Plant height	Biomass
Herbicide	(ml kg ⁻¹ seed)	WAT ^a	4 WAT	6 WAT	(cm plant ⁻¹)	$(g 3 plants^{-1})$
No herbicide	0	-	-	-	72	10 AB^{c}
	0.5	1	5	1	73	11 AB
	1	1	1	3	70	12 AB
	1.5	1	1	1	74	12 AB
	2	1	2	4	69	10 AB
	2.5	2	1	2	72	13 A
	3	1	3	1	70	10 AB
quinclorac +	0	16	14	9	77	10 AB
thiobencarb	0.5	21	9	8	76	12 A
(0.336 + 0.336)	1	13	6	4	80	13 A
kg ai ha ⁻¹)	1.5	30	21	15	83	7 B
	2	10	7	10	82	11 AB
	2.5	34	16	13	77	10 AB
	3	15	31	33	75	11 AB
quinclorac +	0	4	7	12	73	13 A
clomazone	0.5	6	6	11	71	11 AB
(0.336 + 1.12)	1	6	3	5	71	12 AB
kg ai ha ⁻¹)	1.5	3	8	10	71	10 AB
	2	4	3	31	76	13 A
	2.5	8	8	14	68	12 AB
	3	3	6	15	71	11 AB
quinclorac +	0	6	8	8	75	12 A
pendimethalin	0.5	5	4	7	76	13 A
(0.336 + 4.48)	1	3	2	7	74	12 AB
kg ai ha ⁻¹)	1.5	8	2	4	79	11 AB
	2	5	3	5	74	9 AB
	2.5	9	10	11	70	11 AB
	3	7	6	11	73	11 AB
^c P-values		0.925	0.245	0.080	0.922	0.021

Table 9. Effect of primary pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury in the greenhouse, second run, Fayetteville, AR, 2021

^bPlant height was measured at 6 WAT (weeks after treatment), one plant per bucket⁻¹ ^cMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP- values for injury were calculated using a beta distribution in SAS; while for tillers, panicle count, and yield they were calculated using a generalized linear mixed model in JMP Pro 16.1.

	Source of variation	Injury, 4 WAT ^a	Injury, 6 WAT	Tillers (count m ⁻¹)	Panicle (count m ⁻¹)	Yield (kg ha ⁻¹)
Silt Loam Soil	Herbicide	0.2255	0.4757	0.0021*	0.1713	0.4873
	Atonik rate	0.0486*	0.0143*	0.016*	0.2413	0.0641
	Herbicide x Atonik rate	0.5576	0.2567	0.0315	0.7296	0.3419
	Herbicide	0.0072*	0.1152	0.0021*	0.0015*	0.9709
Clay Loam Soil	Atonik	0.2035	0.2233	0.2249	0.7935	0.1261
	Herbicide x Atonik rate	0.1356	0.4733	0.2791	0.4336	0.4933

Table 10. ANOVA table for rice response to primary pre-emergence rice herbicides with different doses of Atonik in Rohwer, Southeast Research and Extension Center, Rohwer, AR, 2021.

^cP--values were generated using the SAS programming for injury, while JMP Pro 16.1 used for tillers, panicle count and yield *Abbreviations: significant factor effect at $P(\alpha=0.05)$

Herbicide ^a	Atonik rate	Injury,4	Injury,	Plant height	Tillers	Panicle	Yield
	(ml kg ⁻¹	WAT ^a	6 WAT	(cm plant ⁻¹)	(count m^{-1})	(count m ⁻¹)	(kg ha^{-1})
	seed)	(%)	(%)				
No herbicide	0	-	-	54	298 DEFGH	76	13007
	0.375	56	59	54	371 ABCDE	75	11770
	0.75	70	64	54	369 ABCDE	71	12312
	1.5	50	28	54	237 GH	70	13709
	3	40	43	54	430 AB	76	12524
quinclorac +	0	45	48	54	332 CDEFG	70	12778
thiobencarb	0.375	78	74	54	401 ABC	64	11762
(0.336 + 0.336)	0.75	20	30	76	430 A	83	12408
kg ai ha ⁻¹)	1.5	48	41	55	354 BCDEF	69	11841
	3	23	21	55	430 AB	69	11742
quinclorac +	0	38	28	53	275 EFGH	59	12611
clomazone	0.375	61	55	54	219 H	74	12328
(0.336 + 1.12)	0.75	31	31	53	346 ABCDEF	72	12235
kg ai ha ⁻¹)	1.5	48	49	53	233 H	71	11156
	3	43	45	51	271 FGH	69	11670
quinclorac +	0	64	45	55	418 ABC	53	12960
pendimethalin	0.375	70	60	54	442 AB	66	11907
(0.336 + 4.48)	0.75	55	50	52	393 ABCD	69	12324
kg ai ha ⁻¹)	1.5	60	55	53	405 ABC	63	11905
	3	56	59	53	420 ABC	76	11889
^c P-values		0.5576	0.2567	0.3750	0.0315	0.7320	0.2020

Table 11. Effect of primary pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury in silt loam soil experiments, Rohwer, AR, 2021.

^aAbbreviations: WAT (weeks after treatment); herbicides were applied immediately after planting

^bMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP-values were generated using the SAS programming for injury, while JMP Pro 16.1 was used for tillers, panicle count and yield

Herbicide ^a	Atonik rate	Injury,	Injury,	Tillers	Panicle	Yield
	(ml kg ⁻¹ seed)	4 WA1°	6 WAI	(count m ⁻)	(count m ⁻)	(kg ha ')
		(%)	(%)			
No herbicide	0	-	-	439	67	10707
	0.375	30	19	457	69	11540
	0.75	26	10	507	67	11670
	1.5	36	9	552	69	11766
	3	45	17	379	78	12009
quinclorac + thiobencarb	0	44	14	403	60	11267
$(0.336 + 0.336 \text{ kg ai ha}^{-1})$	0.375	40	20	373	70	12438
-	0.75	30	12	447	63	11589
	1.5	21	16	412	62	11543
	3	34	19	461	63	10947
quinclorac + clomazone	0	36	18	452	82	11415
$(0.336 + 1.12 \text{ kg ai ha}^{-1})$	0.375	35	17	482	67	11638
	0.75	41	15	450	70	11781
	1.5	29	7	515	86	11487
	3	46	5	466	77	12276
quinclorac + pendimethalin	0	53	30	316	62	11280
$(0.336 + 4.48 \text{ kg ai ha}^{-1})$	0.375	39	24	344	69	11853
	0.75	51	11	404	62	11614
	1.5	43	24	348	61	11195
	3	38	15	409	45	11204
^c P-values		0.1356	0.4733	0.2791	0.4336	0.1847

Table 12. Effect of primary pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury in clay loam soil experiments, Rohwer, AR, 2021

^aAbbreviations: WAT (weeks after treatment); herbicides were applied immediately after planting

^bMeans within a column with different letters are significantly different based on Tukey's protected LSD (α=0.05)

^cP-values were generated using the SAS programming for injury, while JMP Pro 16.1 was used for tillers, panicle count and yield

	Source of variation	Injury, 2 WATª	Injury, 4 WAT	Injury, 6 WAT	Plant height (cm)	Biomass (g)
1 st Run	Herbicide	<.0001	<.0001	0.0021	0.1112	0.0002*
	Atonik	0.0008*	0.2592	0.6567	0.0442*	0.0778
	Herbicide x Atonik	0.0216*	0.0003*	0.1698	0.3376	0.0002*
	-					
	Herbicide	<.0001	<.0001	0.0141*	0.0022*	0.1814
2 nd Run	Atonik	0.1251	0.1292	0.2792	0.4321	0.0108*
	Herbicide x Atonik	0.0329*	0.0238*	0.6623	0.8869	0.0214*

Table 13. ANOVA table for rice response to delayed pre-emergence rice herbicides with different doses of Atonik in the greenhouse, Milo J Shult Agricultural Research and Extension center, Fayetteville, AR, 2022.

^bP-values were generated using a beta distribution in SAS for injury and generalized linear mixed model in JMP Pro 16.1 for Plant height and biomass.

*Abbreviations: significant factor effect at P(α =0.05)

Table 14. Effect of primary delayed pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury, rice height and biomass in the greenhouse, first run, Fayetteville, AR, 2022

Herbicide	Atonik rate (ml kg ⁻¹ seed)	Inj 2 V	ury, VAT	Inj V	ury, 4 VAT	Injury,6 WAT	Plant height (cm)	B (g 1	iomass plants ⁻¹)
No herbicide	0	-	-	-	-	-	58	5	BCDEF ^b
	0.5	13	BA	3	С	3	54	5	BCDEF
	1	13	BA	1	С	1	54	7	ABCDEF
	1.5	14	А	5	BC	3	58	6	ABCDEF
	2	13	А	4	С	5	55	8	ABCDE
	2.5	14	А	1	С	3	55	6	ABCDEF
	3	14	А	1	С	2	52	8	ABCDE
quinclorac +	0	20	D	8	BA	8	56	5	CDEF
thiobencarb	0.5	20	D	8	BA	10	52	5	BCDEF
(0.336 + 0.336)	1	21	DC	10	А	10	56	5	ABCDEF
kg ai ha ⁻¹)	1.5	20	DC	10	BA	8	56	5	BCDEF
	2	13	BC	11	BAC	10	54	5	CDEF
	2.5	19	D	8	BA	15	55	5	DF
	3	20	DC	9	BA	15	55	4	F
quinclorac +	0	13	BA	7	BAC	7	56	5	CDEF
clomazone	0.5	13	BA	1	С	7	53	7	ABCDEF
(0.336 + 1.12)	1	13	А	17	BAC	9	53	5	EF
kg ai ha ⁻¹)	1.5	14	А	4	С	3	53	7	ABCDEF
	2	13	А	2	С	2	53	9	AB
	2.5	13	А	2	С	4	53	7	ABCDEF
	3	14	А	7	BAC	7	53	7	ABCDE
quinclorac +	0	13	BA	5	BC	3	55	5	BCDEF
pendimethalin	0.5	13	BA	3	С	6	55	6	ABCDEF
(0.336 + 4.48)	1	13	BA	3	С	6	55	8	ABCD
kg ai ha ⁻¹)	1.5	14	А	1	С	6	53	6	ABCDEF
	2	13	А	5	BC	2	54	6	ABCDEF
	2.5	13	BA	4	С	2	54	8	ABCE
	3	12	BA	9	BAC	8	54	9	А
P-values		0.0	216	0.	0003	0.1698	0.3376	0.0	002

^bPlant height was measured at 6 WAT (weeks after treatment), one plant per bucket⁻¹ ^cMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP-values were generated using the SAS programming for injury, while JMP Pro 16.1 used for plant height and biomass

	Atonik rate	Injury 2	Injury A	Injury 6	Plant	Biomass
Herbicide	$(ml ka^{-1} seed)$	WAT	WAT	WAT	height	(g 2
	(IIII Kg Seed)	WAI	WAI	WAI	(cm)	plants ⁻¹)
No herbicide	0			-	72	10 AB
	0.5	1 C	1 A	1	73	11 AB
	1	1 C	3 A	1	70	12 AB
	1.5	1 C	4 A	2	74	12 AB
	2	1 C	2 A	1	69	10 AB
	2.5	4 C	4 A	1	72	8 AB
	3	1 C	5 A	2	70	10 AB
quinclorac +	0	35 A	6 A	6	77	10 AB
thiobencarb	0.5	30 BAC	12 A	5	76	12 A
(0.336 +	1	35 BA	6 A	11	80	13 A
0.336 kg ai	1.5	26 BC	10 A	3	83	7 B
ha ⁻¹)	2	25 BC	19 A	2	82	11 AB
	2.5	22 BC	15 A	4	77	10 AB
	3	17 BC	14 A	8	75	11 AB
quinclorac +	0	3 C	3 A	6	73	13 A
clomazone	0.5	5 C	7 A	2	71	11 AB
(0.336 + 1.12)	1	6 C	10 A	3	71	12 AB
kg ai ha ⁻¹)	1.5	2 C	2 A	2	71	10 AB
	2	4 C	4 A	4	76	13 A
	2.5	5 C	3 A	1	68	12 AB
	3	6 C	9 A	3	71	11 AB
quinclorac +	0	25 BC	9 A	3	75	12 A
pendimethalin	0.5	2 C	9 A	1	76	13 A
(0.336 + 4.48)	1	7 C	5 A	6	74	12 AB
kg ai ha ⁻¹)	1.5	1 C	7 A	3	79	11 AB
	2	4 C	1 A	3	74	9 AB
	2.5	7 C	6 A	1	70	11 AB
	3	8 C	8 A	2	73	11 AB
P-values		0.0329	0.023	0.662	0.886	0.021

Table 15. Effect of primary delayed pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury, rice height and biomass in the greenhouse, second run, Fayetteville, AR, 2022

^bPlant height was measured at 6 WAT (weeks after treatment), one plant per bucket⁻¹

^cMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP-values were generated using the SAS programming for injury, while JMP Pro 16.1 used for plant height and biomass.

Table 16. ANOVA table for rice response to delayed pre-emergence herbicides with different doses of Atonik in Rohwer, Southeast Research and Extension center, AR, 2021.

	Source of variation	Injury,	Injury,	Tillers	Panicle	Yield
		4 WAT ^a	6 WAT	(count m^{-1})	(count m^{-1})	$(kg ha^{-1})$
Silt	Herbicide	0.9036	0.8108	0.0021*	0.1713	0.4873
	Atonik rate	0.953	0.6179	0.016*	0.2413	0.0641
	Herbicide x Atonik rate	0.8369	0.6383	0.0315	0.7296	0.3419
	TT 1''1	0.02(1*	0.0040	0.0015*	0.0015*	0.7(2)
	Herbicide	0.0361*	0.2848	0.0015*	0.0015*	0.7626
Clay	Atonik rate	0.3121	0.8116	0.4116	0.7935	0.8798
	Herbicide x Atonik rate	0.0793	0.3419	0.0214	0.4336	0.3604

^aAbbreviations: WAT, weeks after treatment

*Abbreviations: significant factor effect at $P(\alpha=0.05)$

^bP-values were generated using a beta distribution in SAS for injury and generalized linear mixed model in JMP Pro 16.1 for tillers, panicle count and yield.

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Herbicide	Atonik rate (ml kg ⁻¹ seed)	Injury, 4WAT ^b	Injury, 6 WAT	Tillers (count m ⁻¹)	Panicle (count m ⁻¹)	P he (c	lant eight em)	Yield (kg ha ⁻¹)
No herbicide	0	-	-	356	76	50	BC	12789
	0.375	19	25	326	75	51	ABC	11428
	0.75	17	21	308	71	52	ABC	11161
	1.5	14	21	308	70	51	ABC	12647
	3	25	19	296	76	53	AB	12130
quinclorac + thiobencarb	0	22	17	330	70	51	ABC	13591
$(0.336 + 0.336 \text{ kg ai ha}^{-1})$	0.375	15	14	419	64	51	ABC	12058
	0.75	19	14	349	83	54	А	12567
	1.5	13	26	311	69	51	ABC	12623
	3	9	11	318	69	52	AB	11942
quinclorac + clomazone	0	13	13	418	59	51	ABC	12546
$(0.336 + 1.12 \text{ kg ai ha}^{-1})$	0.375	19	16	302	74	52	ABC	13653
	0.75	23	34	256	72	52	ABC	13159
	1.5	15	29	330	71	51	ABC	12723
	3	16	23	388	69	52	ABC	12898
quinclorac +	0	19	26	300	53	51	ABC	13799
pendimethalin	0.375	14	10	247	66	52	ABC	14246
$(0.336 + 4.48 \text{ kg ai ha}^{-1})$	0.75	15	11	317	69	51	ABC	13560
	1.5	20	27	223	63	49	С	13924
	3	21	40	267	76	52	ABC	12935
^C P- value		0.8369	0.6383	0.7448	0.375	0.8	8708	0.8008

Table 17. Effect of primary delayed pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury in silt loam soil experiment, Rohwer, AR, 2021.

^aAbbreviations: WAT (weeks after treatment); Herbicide were applied 2 days after planting.

^bMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cp-values were generated using the SAS programming for injury, while JMP Pro 16.1 used for tillers, panicle count and yield

Herbicide and herbicide rate	Atonik Rate (ml kg ⁻¹)	Injury,4 WAT ^a	Injury, 6 WAT	Tillers (count m ⁻¹)	Panicle (count m ⁻¹)	Plant height (cm)	Yield (kg ha ⁻¹)
No herbicide	0	-	-	300 F ^b	53 D	44	13886
	0.375	32	17	407 AB	79 A	47	13118
	0.75	37	15	394 ABCD	61 BCD	45	13584
	1.5	18	5	347 ABCDEF	65 ABCD	48	13557
	3	47	15	388 ACD	69 ABCD	45	12168
quinclorac + thiobencarb	0	22	4	251 EF	57 D	44	14839
$(0.336 + 0.336 \text{ kg ai ha}^{-1})$	0.375	55	21	331 ABCDE	69 ABCD	46	15249
	0.75	33	22	281 DEF	59 CD	44	16306
	1.5	30	29	384 ABCD	76 AB	44	13136
	3	33	13	348 ABCDEF	62 BCD	44	14791
quinclorac + clomazone	0	41	22	406 AC	66 ABCD	45	12983
$(0.336 + 1.12 \text{ kg ai ha}^{-1})$	0.375	35	7	312 ABCDEF	60 CD	45	13067
	0.75	41	9	274 CDEF	61 BCD	44	12356
	1.5	27	18	380 ABCD	73 ABC	46	13168
	3	37	16	326 ABCDEF	74 ABC	45	13469
quinclorac + pendimethalin	0	61	37	356 ABCDE	57 CD	46	12153
$(0.336 + 4.48 \text{ kg ai ha}^{-1})$	0.375	55	14	372 ABCDE	67 ABCD	45	13524
	0.75	41	14	302 ABCDEF	65 ABCD	45	15153
	1.5	60	34	267 DEF	57 D	44	13409
	3	50	16	277 BEF	76 AB	46	14482
^C P-values		0.0793	0.3419	0.0214	0.4336	0.3604	0.6748

Table 18. Effect of primary delayed pre-emergence rice herbicides and different rates of Atonik seed treatment on rice injury in clay loam soil, Rohwer, AR, 2021.

^bMeans within a column with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^cP--values were generated using the SAS programming for injury, while JMP Pro 16.1 was used to analyze tillers, panicle count and yield

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Appendices

Appendix Table 1. Preliminary data showing interaction of Atonik seed treatment and residual
herbicides on rice yield, Southeast, Rohwer, Research and Extension Center, Rohwer, AR, 2019

Application	Herbicide treatment	Atonik	Yield	Paired t-test
Timing		(ml kg ⁻¹ seed)	$(kg ha^{-1})$	(prob > t)
^a pre-emergence	quinclorac ^a + pendimethalin ^b	0	10308	0 6791
		0.225	10502	0.0781
	quinclorac + clomazone ^c	0	10754	0 7921
		0.225	0.7821	
	quinclorac + thiobencarb ^d	0	10831	0 (90
	-	0.225	10607	0.689
	clomazone	0	10228	0.0075
		0.225	10183	0.8975
	pendimethalin	0	10047	0.7016
	-	0.225	10189	0.7916
	mesotrione	0	9290	0 (259
		0.225	8695	0.6358
^a delayed pre-	quinclorac + pendimethalin	0	9499	0.149
emergence		0.225	9949	
	quinclorac + clomazone	0	10489	0.9286
		0.225	10539	
	quinclorac + thiobencarb	0	10409	0.735
		0.225	10116	
	clomazone	0	10438	0.516
		0.225	10712	
	pendimethalin	0	10110	0.6049
		0.225	9808	
	mesotrione	0	8070	0.7083
		0.225	7880	

^aquinclorac applied at 0.336 kg ai ha⁻¹ ^bpendimethalin applied at 4.48 kg ai ha⁻¹ ^cclomazone applied at 1.12 kg ai ha⁻¹ ^dthiobencarb applied at 0.336 kg ai ha⁻¹

Date	Herbicide	Active ingredient	Application rate (kg ai ha ⁻¹)	Application timing
5/21/2021	Gramoxone	paraquat	1.276	At planting
5/21/2021	Roundup	glyphosate	1.348	At planting
6/4/2021	Newpath ^a	imazethapyr	0.106	3WAP ^b
6/4/2021	Facet	quinclorac	0.426	3 WAP
6/16/2021	Ricestar HT	fenoxaprop-ethyl	0.123	4 WAP
6/17/2021	Newpath	imazethapyr	0.106	4 WAP
6/30/2021	Newpath	imazethapyr	0.106	3-leaf stage
6/30/2021	Londax	bensulfuron-methyl	0.008	3-leaf stage

Appendix Table 2. Maintenance herbicides applied in the preplant experiment to manage weeds, Southeast Research and Extension Center, Rohwer, AR, 2021

^aNon-ionic surfactant was applied at 0.25 % v/v ^bWAP - weeks after planting

Appendix Table 3. Maintenance herbicides applied in the pre-emergence and delayed preemergence experiments to manage weeds, Southeast Research and Extension Center, Rohwer, AR, 2021

Date	Herbicide	Active ingredient	Application rate (kg ai ha ⁻¹)	Application timings
5/21/2021	Gramoxone	paraquat	1.276	At planting
5/21/2021	Roundup	glyphosate	1.348	At planting
6/17/2021	Newpath ^a	imazethapyr	0.106	At planting
6/30/2021	Newpath	imazethapyr	0.106	3-leaf stage
6/30/2021	Londax	bensulfuron- methyl	0.0008	3-leaf stage

^aNon-ionic Surfactant (NIS) was applied at 0.25 % v/v

Month	Total rainfall	Minimum temperature	Maximum temperature
	(mm)	(°C)	(°C)
January	124	10	3
February	160	12	5
March	194	18	9
April	103	22	13
May	93	27	18
June	496	5	10
July	240	10	12
August	75	32	22
September	66	30	19
October	66	24	14
November	65	17	8

Appendix Table 4. Monthly rainfall and temperature data from January through November at the Southeast Research and Extension Center, Rohwer, AR, 2021.

Herbicide	Atonik (ml kg ⁻¹ seed)	Silt loam (/m2)	Clay loam (/m2)
24 D+thioboncarb	0	116	98
2,4 – D + thiobencarb	0.225	116	99
240	0	85	80
2,4-D	0.225	116	91
chlorimuron + flumioxazin +	0	107	61
metribuzin	0.225	89	98
	0	140	91
diuron	0.225	138	87
flumiovorin	0	85	95
Turnoxazin	0.225	85	72
fluridana	0	102	98
liuluolle	0.225	89	104
fluridana + diuran	0	89	146
	0.225	89	95
linuron	0	98	81
indion	0.225	152	85
metribuzin + chlorimuron	0	89	76
	0.225	142	67
our flue afor	0	142	99
oxynuorien	0.225	98	97
saflufonacil	0	107	88
Sanurenden	0.225	95	95
triclonur i clonuralid	0	98	95
	0.225	98	88

Appendix Table 5: List of crop stand oper meter square of silt and clay loam soils in SEREC, Rohwer, AR

Herbicide	Atonik	Pre-clay	Pre-silt	DPRE-clay	DPRE-silt
	0	80	72	79.9	48.95
	0.375	86	63	72.16	53
quinclorac + thiobencarb	0.75	88	79	73.45	68.3
	1.5	80	90	89.05	69.6
	3	91	82	94.07	70.88
	0	82	73	76.03	68.7
	0.375	72	116	70.88	60.55
quinclorac + clomazone	0.75	71	85	69.59	70.43
	1.5	86	58	85.05	82.48
	3	81	79	79.9	56.7
	0	80	79	82.47	51.53
	0.375	95	159	79.9	44.67
quinclorac + pendimethalin	0.75	75	58	67.01	51.55
	1.5	86	57	76.03	51.55
	3	68	80	76.03	47.7
	0	71	91	73.7	84.2
	0.375	85	45	87.63	60.13
No herbicide	0.75	73	89	73.7	91.5
	1.5	77	93	70.88	74.75
	3	75	85	86.34	48.95

Appendix Table 6: List of crop stand of pre-emergence and delayed pre-emergence in silt and clay loam soil of silt and clay loam soils in SEREC, Rohwer.

Chapter 4

Rice response to foliar-applied herbicides with Atonik

Srikanth Kumar Karaikal¹, Isabel Schlegel Werle¹, Matheus Machado Noguera¹,

Gustavo Henrique Bessa de Lima¹, and Nilda Roma Burgos²

¹Graduate Research Assistant, University of Arkansas, Department of Crop, Soil and

Environmental Sciences, Fayetteville, AR, USA

²Professor, University of Arkansas, Department of Crop, Soil and Environmental Sciences, Fayetteville, AR, USA

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Abstract

Biostimulants can improve plant tolerance to abiotic stress, nutritional quality of the crop, growth, and development. Herbicides cause abiotic stress to crops shortly after application, which may affect yield. Experiments were conducted in 2021 at the Southeast Research and Extension Center, Rohwer, AR, and in the greenhouse in 2022 at the Milo J. Shult Agricultural Research and Extension Center, Fayetteville, AR. The objective was to evaluate the effect of Atonik on crop safety of foliar-applied rice herbicides. The field experiment was a split-plot randomized complete block design with four replications with herbicide as the whole plot and Atonik concentration as the sub-plot. There were 11 levels of herbicide treatments (rates are in kg ai ha^{-1}): clomazone + quinclorac (0.336 + 0.336), fenoxaprop-p-ethyl (0.123), quinclorac + fenoxaprop-p-ethyl (0.336 + (0.123), quinclorac + propanil (0.336 + 4.48), penoxsulam + cyhalofop-butyl (0.042 + 0.311), propanil + thiobencarb (0.336 + 3.366), halosulfuron + thifensulfuron (0.050), mesotrione (0.240), florpyrauxifen-benzyl (0.030), and no herbicide. The Atonik treatment had three levels: 0, 0.075, and 0.225 % V/V. The herbicides were applied with recommended adjuvants to 'RT7321FP' rice at V3. Overall, herbicide effect is significant on rice injury at 4 and 6 WAT (weeks after treatment). The effect of herbicide and Atonik on yield was not significant. Numerically, the highest yield $(12880 \text{ kg ha}^{-1})$ was produced when quinclorac + propanil was applied with 0.225% V/V Atonik. The same treatments were also evaluated in the greenhouse in 2022. The Atonik effect was significant on rice injury at 2, 4, and 6 WAT. The interaction effect between herbicide and Atonik was significant at 2 and 6 WAT. Rice injury ranged from 0 to 65% at 2 WAT. The highest injury of 65% was observed when mesotrione was applied without Atonik. At 6 WAT, rice injury ranged from 0 to 55%, the highest injury being with mesotrione applied with 0.075 % V/V Atonik. The effect of herbicide and Atonik was significant on biomass and their interaction was also significant.

The biomass ranged from 2.280 to 29.240 g 3 plants⁻¹. The highest biomass was produced when rice was treated with 0.075% V/V Atonik without herbicide. The lowest was produced when mesotrione was applied with 0.225% V/V Atonik. The experiments indicated that tank mixing Atonik with some foliar herbicides resulted in numerical increase in yield, which could result in economic benefit.

Nomenclature: Atonik®, clomazone, cyhalofop-butyl, florpyrauxifen-benzyl, fenoxaprop-p-ethyl, halosulfuron, mesotrione, *Oryza sativa* L, propanil, penoxsulam, quinclorac, thiobencarb, thifensulfuron. **Key words**: Atonik., biostimulant, foliar herbicides.

Introduction

Rice (*Oryza sativa* L.) is one of the major food crops consumed daily by more than 3.75 billion people worldwide. Rice is a major agricultural commodity in north America although production area is minuscule compared to that in Asia or south America. In 2021, rice was produced in about 1.024 million ha in the US with about 10.74 million tons of production [1]. Arkansas ranks first among the rice-producing states in the US. Thus, it is a major component of the state economy, adding more than \$6 billion in revenue in 2021. To achieve high yields, effective weed management is crucial as weed control is a major obstacle in Arkansas rice production. Weed management is achieved primarily with herbicides, in conjunction with cultural practices. Herbicides and herbicide application costs comprise the largest input in rice production [2]. Most of the rice grown in Arkansas is produced in a drill-seeded, delayed flooding system, with only around 5% being water-seeded [3]. Hence an effective weed control program in Arkansas begins with pre-emergence residual herbicides followed by post-emergence herbicide applications [4]. Pre-emergence herbicides must be applied prior to crop and weed emergence to be effective, unless mixed with a contact foliar herbicide. Popular post-emergence rice herbicides

include propanil, quinclorac, fenoxaprop-p-ethyl, cyhalofop-butyl, halosulfuron, and penoxsulam [5]. Cyhalofop-butyl, and fenoxaprop-p-ethyl, are acetyl CoA carboxylase (ACCase)- inhibiting herbicides used in rice production. These herbicides are recommended for use in rice to control barnyardgrass (*Echinochloa crusgalli*), Amazon sprangletop [*Leptochloa panicoides*) and other grass weeds [6].

Penoxsulam, imazethapyr, bensulfuron-methyl, halosulfuron, and imazamox are a few of the ALS-inhibiting herbicides that are currently registered for use in Midsouth rice production. Weed resistance to ALS herbicides evolved only after widespread Clearfield rice technology adoption. This over-dependence on one mode of action has given rise to ALS-inhibitor-resistance among many weed species including barnyardgrass, weedy rice, yellow nutsedge (*Cyperus esculentus* L.), and rice flatsedge (*Cyperus iria* L.), among others [4].

In the US midsouth, three herbicide-tolerant rice cultivars are: Clearfield, FullPage[™] (tolerant to imidazolinone herbicides), and Provisia[®] (tolerant to ACCase herbicides) [7]. Clearfield[®] technology provides a valuable tool to control the most problematic weed species in the rice production system, weedy rice [8]. IMI herbicides control a broad spectrum of grasses barnyardgrass, weedy rice (*Oryza sativa* L), broadleaf signalgrass (*Urochloa platyphylla*), fall panicum (*Panicum dichotomiflorum*), rice flatsedge (*Cyperus iria*), yellow nutsedge (*Cyperus esculentus*), and broadleaf weeds such as groundcherry (*Physalis angulata*), pale smartweed (*Polygonum lapathifolium*)[9,10]. For optimization of the existing technology and to control problematic weeds in rice production, RiceTec released rice hybrids (Fullpage[™]) that are tolerant to imidazolinone herbicides such as imazamox and imazethapyr herbicides [11]. This system provides a better weed control program in rice production because FullpageTM cultivar allows the use of two ALS inhibiting herbicides: PrefaceTM (imazethapyr) and PostscriptTM (imazamox)

herbicides. PrefaceTM tackles troublesome rice weeds such as red, weedy, feral rice and barnyardgrass. PrefaceTM has post-mergence and residual activity. PostscriptTM can be applied post-emergence or post-flood.

Despite being deemed safe to crops, herbicides still cause oxidative or physiological stress to plants. The exposure of plants to abiotic stress, including herbicide stress, may result in excessive production of reactive oxygen species (ROS). Among the ROS generated in plant cells are singlet oxygen ($^{1}O_{2}$) superoxide (O_{2}^{-}), hydroxyl radicals (OH⁻¹), and hydrogen peroxide (H₂O₂) [12,13] Environmental stresses (drought, heat, freezing, flooding) also cause physiological stress. To mitigate the detrimental effects of abiotic stress, biostimulants can be used to optimize ROS homeostasis in different crops [14]. Plant biostimulants, also known as agricultural biostimulants, when applied at the correct dose and timings, can increase tolerance to abiotic and biotic stress [15].

Biostimulants are substances, including microorganisms, that are applied to plants, seeds, soil, or any other growing media to enhance nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, growth, and harvested crop quality [16]. Biostimulants are not classified as fertilizers but can complement the benefits of fertilizers. Biostimulants are not pesticides [17]. Biostimulants are more frequently used in most of the European nations. The global market for biostimulants is projected to reach USD 6.79 billion by 2030 [18]. One such product is Atonik (Asahi Chemical Manufacturing Company Ltd, Japan). It contains three nitrophenolates: sodium 5-nitroguaiacolate (NaC7H6NO4) 1.25 g L⁻¹, sodium ortho-nitrophenolate (NaC6H4NO3) 2.5 g L⁻¹, and sodium para-nitrophenolate (NaC6H4NO3) 3.75 [19]. Nitrophenolates are common secondary compounds in plants that enhance plant growth by stimulating the activity of antioxidants. Among the antioxidant enzymes the superoxide dismutase (SOD), and ascorbate peroxidase (APX) enzymes

protect cells from oxidative damage due to ROS, which are produced from normal physiological functions such as harvesting light during photosynthesis or, to a fatal extent, from herbicide action.

Preliminary studies with Atonik and foliar rice herbicides were conducted in 2018 and 2019 at the Southeast Research and Extension Center, Rohwer, Arkansas. One study showed that foliar application of Atonik (0.075 % v/v) with quinclorac increased the yield of rice 'Diamond'. The application of Atonik with topramezone at 0.0184 kg ai ha⁻¹ (not labeled in rice) significantly increased rice yield in 2018, but not in 2019 (Appendix Table 1). Slight improvements in rice yield were also observed with the mixture of Atonik and fenoxaprop-p-ethyl. However, adding Atonik to quinclorac + clomazone significantly reduced yield in 2018. Another study showed that spray application volume could change the response of rice to Atonik mixture with some foliar herbicides. Adding Atonik to quinclorac + clomazone or quinclorac + propanil significantly increased yield when applied at 187 ha⁻¹, but not at lower application volumes (Appendix Table 1). Across two years, quinclorac benefited significantly from the addition of Atonik when the herbicide was applied at 187 L ha⁻¹. Several aspects of the use of Atonik with herbicides on rice need to be investigated further. There is scarce information on the effects of post-emergence application of biostimulants with herbicides, especially in rice. Therefore, this research was conducted to determine if Atonik will reduce rice injury from foliar applied rice herbicides and improve rice yield.

Materials and Methods

Greenhouse experiment:

Greenhouse experiments were conducted from January 2022 to July 2022 at the Milo J Shult Agricultural Research and Extension Center (SAREC), Fayetteville, Arkansas (36° 5'54.907" N,
94° 10'42.94" W) to evaluate the effect of biostimulant (Atonik) on rice response to foliar-applied herbicides. The greenhouse conditions were maintained as follows: average temperature was 27.5 \pm 4 day/night, relative humidity of 60 % (Hobo, Onset. Bourne, MA, USA) and with a 14 h photoperiod using high-pressure sodium lamps, $1000 \,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ average photosynthetic photon flux density (PPFD). Silt loam soil with a pH of 7 and 1.6 % organic matter, was passed through a 3-mm sieve. The soil chemical properties of this are in Table 1. FullpageTM ('RT7321FP') hybrid rice was used. Fourteen seeds were planted at a depth of 2.5 to 3.8 cm in each pot (20 cm top diameter). At 10 to 12 days of planting, seedlings were thinned to four plants per pot. The pots were watered as needed. This experiment consisted of 33 treatments, with 11 levels of herbicide treatments: clomazone + quinclorac, fenoxaprop-p-ethyl, quinclorac + fenoxaprop-p-ethyl, quinclorac + propanil, penoxsulam + cyhalofop-butyl, propanil + thiobencarb, halosulfuron + thifensulfuron, mesotrione, florpyrauxifen-benzyl, and no herbicide (Table 2). The 11 herbicide treatments, except mesotrione, are labelled for rice. The Atonik treatment had three levels: 0, 0.075, and 0.225 % v/v. Experiments in the greenhouse were arranged in a split-plot randomized complete block design with four replications and conducted twice. The main factors were herbicide and Atonik concentration. Herbicide was the whole plot, and Atonik concentration was the subplot. The herbicide and Atonik were applied when rice was at two-three leaf stage and 28 to 31 cm tall, averaged across treatments. The treatments were applied in a spray chamber with a flat fan spray nozzle (1100067) (Teejet spray nozzles; Spraying Systems Co., Wheaton, IL) delivering 187 L ha 1 at 269 kPa⁻¹.

Herbicide injury to the seedlings was visually evaluated at 2, 4 and 6 weeks after treatment (WAT) using a scale of 0 to 100, where 0 indicates no control and 100 indicates plant death [20]. The height of tallest plant bucket⁻¹ was measured at 6 WAT. At this time, three plants were

harvested, oven-dried at 65 °C for 3 to 4 days and weighed. One plant was cultured to maturity. At harvest, panicles were counted only from the first run of the experiment. Plants produced mostly unfilled grains during the second run as the plants were grown outdoors and the reproductive stage coincided with the hottest period of the summer. Thus, yield data was not obtained during the second run.

Data were checked using the distribution platform within JMP Pro 16.1 (SAS Institute Inc, Cary, NC). Injury data were analyzed as a beta distribution using ANOVA with SAS 9.4 using PROC GLIMMIX (Gbur et al., 2012). Plant height, biomass, panicle count and yield data were analyzed using the GLIMMIX model add-in JMP Pro 16.1. All data were subjected to analysis of variance (ANOVA), and significant treatment means were separated using Tukey's Kramer (α = 0.05). Each run of the experiments was analyzed separately because of significant interaction between repetition and treatments.

Field experiment

The field experiment was conducted in 2021 at the Southeast research and Extension Center, Rohwer, Arkansas (37°12' 49.272" S, 142° 34' 4.758" E). The soil type is silt loam soil with 1.6% organic matter and a pH of 6.8 (Table 2). 'RT7321FP' rice was drill seeded on May 26, 2021. The herbicide trade names, active ingredient, and manufacturer information are provided in Table 2. Monthly rainfall and temperature data are listed in Appendix Table 4.

The experimental design was a split plot with randomized complete blocks with four replications with herbicide as a whole plot factor and Atonik concentration as the subplot factor. This experiment consisted of 33 treatments with 11 herbicide combinations and three Atonik rates as specified in the greenhouse experiment. The plot dimensions were 1.5 X 4.8 m. The long grain

FullpageTM ('RT7321FP') hybrid rice was drill-seeded, using a planter with 8 drill rows spaced 19 cm apart. The plots were 4.876 m long. Rice was planted on May 26, 2021, and herbicides were applied on June 28 at three-leaf stage of rice using a CO₂-pressurized backpack sprayer attached to a hand-held spray boom with three 8002 flat fan nozzles (Teejet, Glendale Heights, IL) spaced 45.72 cm apart calibrated to deliver 93 L ha⁻¹ at 206 kPa⁻¹. The field was flooded 5 days after treatment application (30 days after planting). The crop was managed culturally according to the University of Arkansas System Division of Agriculture recommendations (Appendix Table 2). Plots were kept weed-free throughout the growing season using conventional postemergence herbicides, not among the treatments, blanket-sprayed over the whole field.

The visible rice injury was evaluated at 4 and 6 WAT. All treatments were compared to the non-treated check (no herbicide and no Atonik). Plant height was measured at 6 WAT, from 6 plants plot⁻¹. Tillers and number of panicles were counted from a meter length of row. Rice was harvested on October 17, 2021, using a Winter Steiger small plot combine and rough rice yield was adjusted to 12% moisture.

All data were analyzed using JMP Pro 16.1 (SAS Institute Inc., Cary, NC). Herbicide, Atonik and their interactions were considered fixed effects. The block, block X herbicide, and block X Atonik were included in the model as random effects. All data were subjected to analysis of variance (ANOVA), and treatment means were separated by Tukey-Kramer protected LSD test at $\alpha = 0.05$. Injury data were analyzed using PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, N.C,), and means were separated using Tukey-Kramer (LSD) (P=0.05). Plant height, panicle count and yield were analyzed using the fir-model platform in JMP Pro 16.1.

Results

Greenhouse run 1:

In the first run, the two-way interaction between herbicide and Atonik was significant on rice injury at 2 and 4 WAT and on biomass, but not on panicle count and yield. (Table 3). The main effect of herbicide was significant on all variables evaluated. At 2 and 4 WAT, except with mesotrione treatment, all treatment combinations caused less than 20% visible injury (Table 4). Mesotrione, without Atonik, caused the highest injury of 60 and 51%, averaged across Atonik treatments. With the highest concentration of Atonik (0.225 % v/v), mesotrione caused 45 and 35% injury, at 2 and 4 WAT, respectively. By 6 WAT, rice injury was generally <5%, but injury with mesotrione remained high at 35%. All other treatments had less than 10% injury. Overall, mesotrione caused the highest injury to rice at all the evaluation times. Rice has low tolerance to mesotrione.

The main effects of herbicide and Atonik on biomass were significant; the interaction effect between herbicide and Atonik concentration was also significant (Table 3). The highest biomass (30 g for 3 plants) was produced from nontreated rice. The application of Atonik alone did not increase rice biomass. Rice treated with quinclorac + propanil; or quinclorac + clomazone produced higher biomass when mixed with Atonik compared to without Atonik (Table 4).

Effect of Atonik on panicle count and yield

The main effect of herbicide on panicle count and yield was significant, but all other factor effects were not (Table 3). Among the treatment combinations, quinclorac + propanil + Atonik produced 14 panicles per plant, which is 28 % higher than the control. Following that, mesotrione + Atonik (0.225%), and halosulfuron + COC both produced 13 panicles per plant. There is a direct relationship between panicle count and yield. Even though mesotrione caused the highest injury to rice, this treatment produced the highest grain yield (14 g per plant), among all the treatment combinations.

Greenhouse run 2:

In the second run, there was no interaction effect between the two factors; however, herbicide effect was significant on all the injury evaluations and on biomass (Table 3). Injury symptoms were visible one week after treatment application. As in the first run, mesotrione caused the highest injury among all herbicide treatments at all three evaluations regardless of Atonik concentration. At all evaluations, the injury from mesotrione was > 60% (Table 5). All other treatments caused < 20% injury to rice.

The two-way interaction between herbicide and Atonik was significant on biomass (Table 3). The highest biomass of 30 g was recorded when rice was treated with halosulfuron was treated with rice (0.225 % v/v) and without Atonik treatment 40% less biomass was produced under the same herbicide.

Field Experiment:

The main effect of herbicide was significant on rice injury and plant height; and the twoway interaction between herbicide and Atonik was significant on panicle count (Table 6). The twoway interaction between herbicide and Atonik application on rice injury was not significant at any evaluation time; however, the main effect of herbicide was significant (Table 6). At 2 WAT, injury symptoms were visible in most plots treated with herbicide, as expected (Table 7). The injury level was less than 15% with rice herbicides but was high (35 to 50%) with mesotrione. The addition of Atonik numerically reduced injury from clomazone + quinclorac, cyhalofop-butyl + penoxsulam, and florpyrauxifen-benzyl. Injury from these herbicides were reduced to below 10% with Atonik. However, the addition of Atonik, at the rates tested, did not alleviate the injury from mesotrione. At the later evaluation timings, rice injury was almost zero in all treatments, except with that of mesotrione, which hovered around 20%. It was noteworthy that the high rate of Atonik reduced rice injury from mesotrione to 14% at 4 WAT.

There were no significant interaction effects on plant height, but the main effect of herbicide was significant (Table 6). At 6 WAT, the mean plant height for herbicide treatments ranged from 85 to 127 cm averaged across Atonik concentrations. The mean plant height across herbicide treatments without Atonik was slightly higher than that in the Atonik-treated plots. This height differential with Atonik was not observed in preliminary research (data not shown).

The interaction effect between herbicide and Atonik concentration was significant on panicle count and none of the main effects were significant (Table 6). The mean panicle count ranged from 44 to 63 panicles m⁻¹ across herbicide treatments (Table 7). The highest number of panicles (69 panicles m⁻¹) was recorded from the quinclorac + fenoxaprop-p-ethyl + without Atonik and the lowest (45 panicles m⁻¹) was recorded from the mesotrione + Atonik (0.225 % v/v) treated plot.

The two-way interaction was not significant on rough rice yield (Table 6). Generally, treatments containing higher concentration of Atonik (0.225 % v/v) produced numerically higher yield in general, compared to other treatments. In this experiment, Atonik was applied with various herbicides and compared with herbicide alone. The highest yield (13491 kg ha⁻¹) was obtained from plots treated with quinclorac + propanil without Atonik, while the lowest yield was from the nontreated check (no herbicide and no Atonik) (Table 7). However, a preliminary test in 2020, the highest yield of 13911 kg ha⁻¹ was obtained from plots treated with clomazone and 0.2 % v/v

Atonik (data not shown). Overall, Atonik + herbicide treatments produced numerically higher yield compared to the herbicide alone. Herbicide treatments that benefitted from the Atonik application were clomazone + quinclorac, cyhalofop-butyl + penoxasulam, florpyrauxifen-benzyl, mesotrione, propanil + thiobencarb and no herbicide.

Discussion

Biotic and abiotic stress can reduce the yield of agricultural crops by 13 - 91 % across the globe. Crop yield loss from various stress factors are listed in Table 8. To reduce the yield loss caused by abiotic stress, biostimulants maybe helpful [21]. Biostimulants are derived from a variety of sources, resulting in significant variations in their chemical profiles, not only quantitatively, but also qualitatively [22], which results in wide variety of product performance. The positive effect of biostimulants on the growth and development of cereal crops such as wheat, corn, and barley have been reported [23]. Therefore, this research focused on the application of Atonik with selected foliar herbicides in rice. The evaluations include injury, plant height, panicle count, and rice grain yield were investigated in plots that are treated with and without Atonik.

In Arkansas, the most common foliar herbicides in rice production include quinclorac, bensulfuron-methyl, imazethapyr, imazamox, penoxsulam, bispyribac, clomazone, halosulfuron, cyhalofop-butyl, florpyrauxifen-benzyl, and propanil [24,25]. The efficacy of foliar herbicides is dependent on several factors including temperature, relative humidity, the light, wind, soil moisture, rain, diluent concentration, and plant condition [26]. The herbicides used in these experiments have different modes of action and were used because the mode of action could conceivably influence the activity of Atonik biostimulant, induction of plant defenses, and the effects of herbicide stress.

Due to excessive rainfall, injury at 2 WAT data was not collected (Appendix Table 3). Rice injury (35%) was highest at 4 and 6 WAT with mixture of mesotrione + Atonik. Mesotrione is labeled for use in

corn, sweet corn, field corn, and yellow popcorn but not rice [27]. Which provides pre-emergence and post-emergence control of all the important broadleaved weeds in maize[28]. However, mesotrione cannot be used to control weeds in other crops which are sensitive to this herbicide. The rice injury was slightly higher in the greenhouse than in field and is likely due to the fact that greenhouse-grown plants are not hardened. There was no effect of Atonik application on reducing damage caused by mesotrione on rice. However, injury from WSSA group 6 (propanil), group 8 (thiobencarb), group 13 (clomazone), group 4 (quinclorac), and group 12 (halosulfuron) were less than 15% except group 4 (florpyrauxifenbenzyl) which caused 21% injury even with the highest concentration of Atonik. These results were consistent with the study on rice response to florpyrauxifenbenzyl at labeled rate [29].

It is important to note that most biomass collected from plants treated with herbicides including mesotrione was lesser than the non-treated, which was contradictory to the field experiment. However, in the field, plants had several months to recover from herbicide injury, while plants in the greenhouse had only one month of growth before biomass was collected. These results were consistent with the previous experiment conducted with only two concentrations of Atonik (0 and 0.225% v/v) and different foliar rice herbicides. Numerous studies showed positive impact of biostimulants on the growth and development of cotton, wheat, and other crop plants [30,31]. Several factors affect yield, including the type and dose of biostimulant used, the method of application and the variety of crop [32]

The mixture of herbicides and the Atonik concentrations, could affect rice yield differently. Furthermore, regardless of the Atonik concentration used, mixed application with herbicides resulted in lower rice yield than when Atonik was applied separately. Overall, experiments showed that foliar application of herbicide and Atonik biostimulant mixture had no significant impact on rice yield. Similar results were obtained when biostimulant was sprayed on wheat, corn, oats and sugarbeet [33,34]. However, foliar application of Atonik alone on sunflower (Helianthus annuus) and horsebean (Vicia faba var. equina.) resulted in a slight increase in yield [35,36]. Future research is needed to evaluate rice response to foliar herbicides with higher Atonik concentrations starting from 0.3 % v/v. Additionally, further research should be conducted to determine if other rice hybrids respond similarly the cultivar used in these experiments.

Conclusions

Results from these experiments indicate that foliar application of Atonik did not reduce the herbicide injury but slightly improved the rough rice yield. Programs containing mesotrione are superior in producing rough rice yield compared to nontreated rice. Although maximum injury was observed immediately after mesotrione application irrespective of Atonik concentration, but an increasing yield was observed with increasing Atonik concentration. However, the highest yield produced from quinclorac + propanil without Atonik treatment. Additional research is needed to determine at what Atonik concentration do we obtain higher yields than untreated rice when foliar herbicides are applied.

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Tables and Figures

Location	Soil type	\mathbf{P}^{H}	Р	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	В	Sand	Silt	Clay
]	ng kg	g ⁻¹						- (%) -	
SEREC ^a , Rohwer	Silt	6.7	84	186	1326	272	5.8	6.4	235	106	4	1.7	0.4	17.3	67.7	15
SAREC ^b , Fayetteville	Silt	7.1	49	103	1073	40	7.1	7.4	88	213	2.2	1.3	0.4	27.1	59	14

Table 1: Selected soil chemical properties at the sites where the experiments were conducted in 2021 and 2022.

^aAbbreviations: SEREC – Southeast Research and Extension Center, Rohwer

^bAbbreviations: SAREC – Milo J. Shult Agricultural Research and Extension Center

Herbicide treatments ^a	Trade name	Application rate (kg ai ha ⁻¹)	Manufacturer
$clomazone + quinclorac + COC^{*b}$	Command + Facet	0.336 + 0.28	FMC corporation 2929 Walnut Street Philadelphia, PA; BASF
cyhalofop-butyl + penoxsulam + COC*	Rebel EX	0.31 + 0.043	Corteva Agri Sciences, Indianapolis, IN
fenoxaprop-p-ethyl	Ricestar HT	0.123	Bayer CropScience, Research Triangle Park NC.
florpyrauxifen-benzyl + MSO	Loyant	0.029	Corteva Agri Sciences, Indianapolis, IN
halosulfuron + thifensulfuron + COC^*	Permit plus	0.067	Gowan, Yuma, AZ
mesotrione + COC^*	Callisto	0.236	Syngenta Crop Protection, Inc.
propanil + thiobencarb + COC^*	RiceBeaux	4.48 + 0.28	UPL
propanil + thiobencarb; halosulfuron	Ricebeaux + Permit plus	4.48 + 0.28 + 0.067	UPL; Gowan, Yuma, AZ
quinclorac + fenoxaprop-p-ethyl	Facet + Ricestar HT	0.28 + 0.123	BASF Corporation 26 Davis Drive,
			Research Triangle Park, NC; Bayer
			CropScience, Research Triangle Park
			NC.
quinclorac + propanil	Facet + Stam M4	0.28 + 4.48	BASF Corporation 26 Davis Drive,
			Research Triangle Park, NC; Corteva
			Agri Sciences, Indianapolis, IN

Table 2: Herbicides tested with Atonik, applied on silt loam soil, Southeast Research and Extension Center, Rohwer, AR, 2021

^aHerbicides and Atonik were applied at two- three leaf stage, with the respective recommended adjuvants in 93 L ha⁻¹ of spray volume ^b *Abbreviations: COC (Crop Oil Concentrate are applied at 1% v/v)

Table 3: Analysis of variance on the effect of foliar herbicides and Atonik on rice injury, height, biomass, panicle count and rough rice yield, in the greenhouse, Milo J Shult Research and Extension Center, Fayetteville, AR, 2022

	Source of variation	Injury, 2 WAT (%)	Injury, 4 WAT (%)	Injury, 6 WAT (%)	Biomass (g)	Panicle (count plant ⁻¹)	Yield (g plant ⁻¹)
Run1	Herbicide	<.0001*	0.0017	<.0001*	<.0001*	0.0059*	0.0052*
	Atonik rate	<.0001*	0.0006	0.0101*	0.1574	0.2148	0.1635
	Herbicide*Atonik rate	0.001*	<.0001*	0.1577	0.0019*	0.8308	0.3083
	Herbicide	<.0001*	<.0001*	<.0001*	<.0001*	-	
Run 2	Atonik rate	0.608	0.7751	0.2401	0.2295		
	Herbicide*Atonik rate	0.4856	0.1058	0.8472	0.0065*	_	

^aAbbreviations: WAT, weeks after treatment

^bAsterisks (*): indicates significant treatment effects

Table 4. Effect of Atonik tank-mixed with herbicides on rice 'RT7321FP' injury and biomass in the greenhouse run 1, Fayetteville, AR, 2022.

Harbicidas	Atonik rate		Injury,	Injı	ıry,	Injury, 6	Bio	omass
Herbicides	(%v/v)	2	WAT ^a (%)	4 WA	T (%)	WAT (%)	(g 3 j	plants ⁻¹)
No herbicide	0	5	CED ^c	10	BA	2	29	А
	0.075	1	E	6	BA	2	26	ABC
	0.225	3	ED	15	BA	6	29	А
clomazone + quinclorac + coc	0	3	ED	2	BA	2	26	ABC
	0.075	3	ED	1	В	2	24	ABC
	0.225	3	ED	8	В	2	27	AB
cyhalofop-butyl + penoxasulam + coc	0	5	CED	5	BA	2	2	ABCD
	0.075	1	E	1	В	2	26	ABC
	0.225	2	E	3	В	7	26	ABC
fenoxaprop-p-ethyl	0	14	CBD	3	BA	1	20	ABCD
	0.075	8	CEBD	6	В	2	20	ABCD
	0.225	4	CED	3	В	4	20	ABCD
florpyrauxifen-benzyl + MSO	0	18	В	3	BA	4	18	BCD
	0.075	15	CB	2	В	1	16	BCDE
	0.225	21	В	19	В	15	6	EF
halosulfuron + coc	0	1	E	6	BA	4	27	ABC
	0.075	1	E	2	BA	2	25	ABC
	0.225	2	E	10	В	9	20	ABCD
mesotrione $+ \cos$	0	60	А	50	А	35	13	DEF
	0.075	49	А	45	BA	30	3	F
	0.225	45	А	44	BA	34	2	F
propanil + thiobencarb + coc	0	13	CBD	1	BA	3	25	ABC
	0.075	14	CEBD	6	BA	2	22	ABCD
	0.225	14	CBD	4	BA	2	27	ABC
propanil + thiobencarb; halosulfuron + coc	0	9	CED	9	BA	5	22	ABCD
	0.075	4	CBD	1	В	3	24	ABCD
	0.225	4	CED	5	В	4	21	ABCD

Table 4. (Cont.)

Herbicides ^b	Atonik rate (%v/v)	Injury, 2 WAT ^a (%)	Injury, 4 WAT (%)	Injury, 6 WAT (%)	Biomass (g)
quinclorac + fenoxaprop-p-ethyl	0	20 B	16 BA	2	16 CD
	0.075	10 CEBD	3 B	4	20 ABCD
	0.225	2 E	2 B	4	23 ABCD
quinclorac + propanil	0	9 CEBD	2 BA	8	24 ABCD
	0.075	3 ED	6 B	4	19 ABCD
	0.225	3 ED	3 B	2	26 AB
^d P-values		0.001*	<.0001	0.1577	0.0019

^aAbbreviations: WAT, weeks after treatment

^bHerbicides and Atonik were applied at two-three leaf stage

^cMeans within a with different letters are significantly different based on Tukey's protected LSD (α =0.05)

^dP-values were generated using the generalized mixed model in SAS for injury and using JMP Pro 16.1 for biomass

Herbicides ^b	Atonik rate (% v/v)	Injury, 2 WAT ^a (%)	Injury, 4 WAT (%)	Injury, 6 WAT (%)	B (g 3	iomass plants ⁻¹)
No herbicide	0	6	3	2	18	ABCD ^c
	0 075	1	2	3	21	ABC
	0.225	1	1	4	17	BCD
clomazone + quinclorac + coc	0.225	2	5	6	22	ABC
elomazone + quinciorae + coc	0 075	3	5 7	3	$\frac{22}{20}$	ABCD
	0.225	5	7	4	17	BCD
cyhalofop-butyl + penoxasulam + coc	0.225	3	12	10	28	AB
	0.075	2	7	4	27	AB
	0.225	6	9	6	27	AB
fenoxaprop-p-ethyl	0	12	9	8	13	CDE
	0.075	8	3	8	20	ABCD
	0.225	4	3	10	18	ABCD
florpyrauxifen-benzyl + MSO	0	8	3	8	18	ABCD
	0.075	7	8	9	21	ABC
	0.225	7	3	5	30	AB
halosulfuron $+ \cos$	0	5	9	9	30	А
	0.075	6	3	9	18	ABCD
	0.225	3	10	8	25	ABC
mesotrione $+ \cos$	0	75	71	73	12	CDE
	0.075	79	71	68	0	DE
	0.225	83	76	79	1	Е
propanil + thiobencarb + coc	0	4	4	5	23	ABC
	0.075	8	10	6	23	ABC
	0.225	6	8	7	24	ABC
propanil + thiobencarb; halosulfuron + coc	0	5	2	4	23	ABC
	0.075	2	4	3	26	AB
	0.225	4	3	8	28	AB
quinclorac + fenoxaprop-p-ethyl	0	8	2	3	21	ABC
	0.075	10	4	2	16	BCDE
	0.225	4	7	6	21	ABCD
quinclorac + propanil	0	3	3	7	20	ABC
	0.075	6	7	7	20	ABCD
	0.225	3	8	8	21	ABC
^d P-values		0.4856	0.1058	0.8472	().0065

Table 5. Interaction effect of foliar-tank mix herbicide and Atonik rates on rice ('RT7321FP') injury of the Run 2, greenhouse, Fayetteville, AR, 2022.

^aAbbreviations: WAT, weeks after treatment

^bHerbicides and Atonik were applied at two-three leaf stage. MSO = 0.5 pints acre⁻¹;COC = 1% v/v

^cMeans within a with different letters are significantly different based on Tukey's protected LSD (α =0.05).

^dP-values were generated using the generalized mixed model in SAS for injury and using JMP Pro 16.1 for biomass.

	Source of variations	Injury, 4 WAT ^a (%)	Injury, 6 WAT (%)	Plant height (cm)	Panicle (count m ⁻¹)	Yield (kg ha ⁻¹)
Silt loam soil	Herbicide	<.0001*	<.0001*	0.0355*	0.142	0.5906
	Atonik rate	0.0868	0.2648	0.394	0.8019	0.7481
	Herbicide* Atonik rate	0.614	0.0832	0.3364	0.0171*	0.1272

Table 6. Analysis of variance on the effect of foliar herbicide and Atonik on rice injury, plant height, biomass, panicle count and rough rice yield, Southeast Research and Extension Center

^aAbbreviations: WAT, weeks after treatment

^bAsterisks(*): indicates significant treatment effects

Herbicide ^b	Atonik rate	Injury, 4 WAT ^a (9)	Injury, 6 WAT (0)	Plant	Panicle	Yield $(kg ha^{-1})$
	(% V/V)	WAI [®] (%)	WAI (%)	(cm)	$(no plant^{-1})$	(kg lla)
No harbicida	0	12 PC ^c	2	00		10341
No herbicide	0 075	13 DC 12 DC	2	99	4J 56	10341
	0.075		5	99	50	11230
1	0.225	9 C	l c	99	01	12095
clomazone + quinclorac + coc	0		5	98	56	12026
	0.075	7 C	l	100	57	12468
	0.225	8 C	5	99	60	12079
cyhalofop-butyl + penoxasulam + coc	0	11 BC	7	98	60	11960
	0.075	5 C	1	99	50	12812
	0.225	3 C	5	100	61	11632
fenoxaprop-p-ethyl	0	9 C	2	101	60	12397
	0.075	11 C	1	100	54	12389
	0.225	7 C	3	100	55	12236
florpyrauxifen-benzyl + MSO	0	13 BC	3	99	49	12106
	0.075	6 C	5	99	54	12267
	0.225	5 C	3	100	46	12789
halosulfuron $+ \cos$	0	3 C	2	102	45	12445
	0.075	5 C	1	102	47	11546
	0.225	3 C	1	102	54	12381
mesotrione $+ \cos$	0	46 BA	20	86	48	11844
	0.075	50 A	20	85	66	12132
	0.225	35 BAC	14	92	45	12225
propanil + thiobencarb + coc	0	8 C	8	99	63	11900
1 1	0.075	8 C	5	100	49	12491
	0.225	6 C	4	100	48	11786
propanil + thiobencarb; halosulfuron + coc	0	10 C	3	100	58	12458
	0.075	10 BC	6	99	54	12353
	0.225	12 C	2	98	50	12700

Table 7: Effect of foliar tank-mix herbicides and Atonik rates on rice ('RT7321FP') panicle count and yield in SEREC, Rohwer, AR,2021.

Table 7. (Cont.)

Herbicide ^b	Atonik rate	Injury, 2	Injury, 4	Injury, 6	Biomass (g)	Yield
	(% v/v)	WATa (%)	WAT (%)	WAT (%)	_	(kg ha^{-1})
quinclorac + fenoxaprop-p-ethyl	0	6 C	3	128	69	12550
	0.075	2 C	1	100	56	12234
	0.225	2 C	3	99	60	12389
quinclorac + propanil	0	2 C	1	100	58	13491
	0.075	11 BC	4	97	55	12764
	0.225	4 C	1	99	61	12386
^d P-values		0.6	0.0832	0.3364	0.0171	0.1272

^aAbbreviations: WAT, weeks after treatment

^bHerbicides and Atonik were applied at two-three leaf stage.

^cMeans within a with different letters are significantly different based on Tukey's protected LSD (α =0.05).

^dP-values were generated using the generalized mixed model in SAS for injury and for plant height, panicle count and yield analyzed using JMP Pro 16.1

Stresses	Crop	Stress Type	Yield loss (%)	References
	Wheat	Pathogens, Viruses, animal pests and weed	16,3,9 and 23	
Biotic	Rice	Animal, weeds and pathogen pests	37,25 and 13	- [37].
	Maize	Animal, pathogen, and viruses	16, 9 and 3	
Abiotic	Cotton, soybean	Temperature	10.3 and 25.60	[38]
	Rice	Drought	70	[39]

Table 8: Important biotic and abiotic stresses limiting productivity of major row crops.

Appendices

Appendix Table 1. Interaction effect of foliar herbicides with Atonik (0.075 % v/v) applied at different application volumes on yield of Diamond rice, Rohwer, AR, 2019.

Herbicide common name	Herbicide rate (kg ai ha ⁻¹)	Atonik (0.075% v/v)			Rice y	ield (kg ha ⁻¹)) ^a	
			5	GPA	10) GPA	20 0	GPA
Check ^b		with	8502	DE ^a	9563	CD	8218	G
		without	8390	DE	9475	DE	8660	G
quinclorac 0.28	0.28	with	9882	ABC	9002	E ab	8886	FG
	0.28	without	8203	DE	10487	ABC	8750	FG
quinclorac +	0.28 + 0.336	with	9414	BCDE	10006	ABCD	9744	BCDE
clomazone		without	9915	ABC	9732	BCD	8590	G
quinclorac +	0.28 + 0.123	with	7894	E	10793	А	9593	CDEF
fenoxaprop-p-ethyl		without	8920	DE	10463	ABC	9066	DEFG
auinalarea propenil	0.28 + 4.48	with	9631	ABCD	10290	ABCD	10189	ABC
quinciorae + propann	0.20 ± 4.40	without	10303	AB	9585	CD	9161	DEFG
topromozono	0.02	with	10560	А	10485	ABCD	9913	BCD
topramezone	0.02	without	10010	ABC	10619	Ab	10598	AB
magatriana	0.21	with	10272	AB	10746	А	11141	А
mesourione	0.21	without	10294	AB	9691	CD	10216	ABC

^aDifferent uppercase letters within a column or lowercase letters within a row indicate significant difference at α =0.05, based on Fisher's t-test.

^bThe whole test was sprayed with maintenance pre-emergence herbicides: clomazone (Command 1.5 pt/A) + quinclorac (Facet 0.76 lb/A). Foliar herbicides were applied 30 d after planting.

Herbicide	Active ingredient	Application rate (kg ai ha ⁻¹)	Application timings
Gramoxone	paraquat	1.254	At planting
Roundup	glyphosate	1.539	At planting
prowl H ₂ O	pendimethalin	0.002	At planting
Newpath ^a	imazethapyr	0.0002	At planting
Newpath	imazethapyr	0.002	3-leaf stage
Newpath	imazethapyr	0.002	3-leaf stage
Londax	bensulfuron-methyl	0.001	3-leaf stage

Appendix Table 2. maintenance herbicides applied in the rice production to manage weeds in the silt loam soil, SEREC, Rohwer, AR, 2021

^aNon-ionic Surfactant (NIS) was applied at 0.25 % v/v

Appendix Table 3. Rainfall (mm), minimum and maximum temperature (°C) history of 2021 from January through November in Southeast Research and Extension Center, Rohwer, AR, 2021.

Month	Total Rainfall	Minimum	Maximum
	(mm)	Temperature (°C)	Temperature (°C)
January	124	10	3
February	160	12	5
March	194	18	9
April	103	22	13
May	93	27	18
June	533	5	10
July	240	10	12
August	75	32	22
September	66	30	19
October	66	24	14
November	65	17	8

General Conclusions

Atonik seed treatment has been proven to be effective in increasing crop productivity during abiotic stress. The addition of Atonik synthetic biostimulant to current rice herbicides would provide marginal improvement in rice yield but it may be economically beneficial to producers. Applying Atonik as seed treatment did not reduce the injury caused by the most used preplant, pre-emergence or delayed pre-emergence herbicides significantly but it had stimulated plant growth and development, particularly recovery. Moreover, biomass accumulation and yield production are stimulated by Atonik. The positive effect of Atonik is not apparent when plants are treated with herbicides.

The optimum Atonik seed treatment appears to be 1.5 ml kg⁻¹ of seed for quinclorac + thiobencarb and quinclorac + clomazone. Below this rate rice tolerance to herbicides was inconsistent and the rate of 3.0 ml kg⁻¹ did not provide any additionally benefit. Additionally, for quinclorac + pendimethalin treatment, the optimum Atonik seed treatment is 2.5 ml kg⁻¹ of seed below this rate, this rate herbicide causes high injury to rice crop. Additionally, > 10% injury was observed across all the Atonik rates with two soil types. Lastly, < 45 % injury to rice was observed with quinclorac + pendimethalin across Atonik rates under adverse rice growing conditions in field, demonstrating the ability of this biostimulant to perform in conditions typical during the best across the U.S. Based on this research and previous literature, the Atonik seed treatment is not best agronomic tool in reducing herbicide stress, but the Atonik seed treatment can stabilize rice yield.

Foliar application of Atonik benefited some rice herbicides in yield production. However, it did not reduce the herbicide stress significantly, including mesotrione, which is not labeled for rice. The yield with mesotrione was almost similar to the non-treated. Based on this research, the optimum Atonik concentration for the most foliar rice herbicide is 0.225% v/v. Future research needs to be conducted across locations and years with higher range of Atonik concentrations between 0.225 and 3 % v/v.