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In Vitro Evaluation of Commercial Fungicides against Some of the Major Soil Borne Pathogens of Soybean

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Abstract

Four Strobilurin, two premix of Strobilurin and Triazole, and one pyrazole-carboxamide foliar fungicides were tested in a modified *in vitro* culture plug technique against *Colletotrichum truncatum* (CT), *Fusarium virguliforme* (FV), *Macrophomina phaseolina* (MP), *Pythium irregulare* (PI), *Rhizoctonia solani* (RS), and *Sclerotinia sclerotiorum* (SS) and three Strobilurin and two premixes against *Septoria glycines* (SG). Under aseptic conditions, a single 6-mm culture plug of actively growing individual fungus was placed inverted on one end inside periphery of 9-cm PDA plates and on the opposite end 6-mm sterilized blotter disc with 50-µl fungicide solution was placed. Tests against SG were by spreading 50-µl spore suspension (1×10^8 spores/ml) on to PDA and placing blotter disc with 50-µl fungicide in the center. During 12-day incubation in 12-h photoperiod, assessed *in vitro* (i) effects of fungicides on growth of pathogens, (ii) sensitivity of pathogens to fungicides and (iii) persistence of fungicide tolerance in pathogens. All the fungicides except Sercadis, significantly ($P<0.05$) reduced radial growth of CT, while Headline EC, Priaxor and Stratego YLD significantly reduced growth of FV, MP, RS and SS. Similarly, Sercadis was effective against RS, and Aproach and Quadris against FV. SG and CT showed significant ($P<0.05$) sensitivity to most of the fungicides, FV, RS and SS showed significant sensitivity by forming inhibition zone between their growth ends and Headline EC, Priaxor and Stratego YLD discs. CT, MP and RS showed significant ($P<0.05$) persistence to all the fungicides that is considered fungistatic effect.

Keywords: Fungicides; Strobilurin; Triazole; Pyrazole-caroxamide; Fungistatic effect; Soybean pathogens; Area under colony growth

Introduction

Soybean [*Glycine max* (L.) Merr.] is the leading oilseed crop produced and consumed in the world. According to Hymowitz et al., [1] as of 2013, soybean was grown in 70 countries with an annual production of 268 million metric tons (mmt). Top eight leading producers of soybean are United States (31%), Brazil (31%), Argentina (19%), China (5%), India (4%), Paraguay (3%) and Canada (2%). As of December 2015, USDA projection of World Soybean Production 2015/2016 is 320.11 mmt it is an increase of 1.11 mmt or a 0.35% compared with 2014 [2].

Worldwide more than 200 pathogens are affecting soybean, of which at least 35 have been reported economically important [3]. Some of the important early season diseases in Iowa, United States are; Phytophthora root rot (*Phytophthora* spp.), Pythium damping off and root rot (*Pythium* spp.), Rhizoctonia root rot (*Rhizoctonia solani*) and sudden death syndrome (*Fusarium virguliforme*). Mid to late season diseases are brown spot (*Septoria glycines*), anthracnose (*Colletotrichum* spp.), sudden death syndrome (*F. virguliforme*), charcoal rot (*Macrophomina phaseolina*), and white mold (*Sclerotinia sclerotiorum*). A comprehensive report of soybean diseases in Argentina, Brazil, Canada, China, India, Japan and United States have been compiled in the latest compendium of soybean diseases and pests [4]. The foliar, stem and root diseases of soybean are important components of yield loss in soybean fields. In Iowa, bacterial leaf blight (*Pseudomonas savastanoi* pv. *glycinea*), frogeye leaf spot (*Cercospora sojina)*, Cercospora leaf blight (*C. kukuchii)*, downy mildew (*Peronospora manshurica*), and Septoria leaf spot or brown spot (*S. glycines*) are present without causing significant impact on yield [5]. However, these diseases do reduce photosynthetic activity in infected leaves by reducing green leaf area [6] and affecting photosynthesis in the asymptomatic area of diseases infection [6,7]. On the other hand yield losses due to stem and root diseases like Rhizoctonia root rot, Pythium and Phytophthora root rot, sudden death syndrome and white mold are up to 35% [8].

According to USDA-NASS [9] fungicides use in soybean has gone up from <1% of the soybean planted acreage in 20 program states (Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, South Dakota, Tennessee, Virginia, and Wisconsin) to 11% of soybean planted acres in 2012. Similarly, quantity of fungicides applied on soybean has gone up from 48 metric ton in 2002 to 490 metric ton in 2012 on annual basis.

Several seed treatment products (chemical and biological) were tested against *Fusarium* spp., *Pythium* spp., *R. solani*, *Phytophthora sojae* [10], and against *S. sclerotiorum* both *in vitro* and field [11]. Also, some specific active ingredients like azoxystrobin [12-14], pyraclostrobin [15], trifloxystrobin [16], thiophanate-methyl [17], prothioconazole [18], and fludioxonil [13,15,19] tested against *Fusarium* spp. There are several such reports of testing seed treatment products against soil borne pathogens in cereals, legumes and oil seeds. Perhaps our approach is similar to [20] to identify potential and alternate use of foliar fungicides as seed treatment. As a first step, *in*

vitro tests of some of the foliar fungicides against major soil borne pathogens of soybean is undertaken.

There are various *in vitro* methods to test efficacy of fungicides, like paper disc-agar diffusion technique [21], food poisoning technique [22], agar-well diffusion technique [23], poison plate tests and spore germination tests [24-26]. In the current study, a modified dual culture plug technique of Rahman et al., [27] was adopted to test foliar fungicides (Strobilurin, premix of Strobilurin and Triazole, and pyrazole-carboxamide) against major soil borne pathogens of soybean (*C. truncatum*, *F. virguliforme*, *M. phaseolina*, *P. irregulare*, *R. solani*, and *S. sclerotiorum* and *S. glycines*). Objectives of the current study were to 1. Assess *in vitro* effects of fungicides on growth of pathogens, 2. Assess *in vitro* sensitivity of pathogens to various fungicides and 3. Assess *in vitro* tolerance of pathogens to fungicides. Eventually, to find an alternate use of these fungicides as potential seed treatment against several pathogens.

Materials and Methods

Soybean pathogens

Iowa field isolates of *Colletotrichum truncatum* (CT), *Fusarium virguliforme* (FV), *Macrophomina phaseolina* (MP), *Pythium irregulare* (PI), *Rhizoctonia solani* (RS), and *Sclerotinia sclerotiorum* (SS) were isolated on potato dextrose agar (PDA) under aseptic conditions, and were maintained on PDA plates at 23 ± 1˚C throughout the study period. *Septoria glycines* (SG) isolate 14Sg1-23 grown on V8 juice medium (with Rifamycin) was collected from Dept. of Crop Sciences, University of Illinois at Urbana-Champaign, Illinois under an USDA permit and was maintained on V8 juice medium. To maintain on V8 juice medium, added 1-ml distilled sterile water to wash off oozing out conidia, rubbed the growth with a disposable Lazy-L-spreaders (Research Products International Corp.) to loosen the conidia. Pipetted 50-µl to a fresh V8 plate and spread with a pre-sterilized polystyrene disposable lazy-L-spreader, incubated at 23 ± 1°C in 12 h light on 12 h off cycle for a week.

Fungicides and stock solution preparation

Foliar fungicides picoxystrobin (Aproach®), fluoxastrobin (Evito®), pyraclostrobin (Headline EC®) and azoxystrobin (Quadris®), pyraclostrobin + fluxapyroxad (Priaxor®), trifloxystrobin + prothioconazole (Stratego YLD®), and fluxapyroxad (Sercadis®) were evaluated against CT, FV, MP, PI, RS, and SS. Whereas, against *S. glycines* only Aproach, Headline EC, Priaxor, Quadris, and Stratego YLD were evaluated. List of fungicides, labeled application rates, active ingredients, group name, FRAC code and manufacturer is provided in Table 1.

Fungicide product	Dilutions used (ml/L)[1]	Labeled rates[2] ml/Ac	Active ingredient (%)	Group name	FRAC* code	Manufacturer
Aproach®	3.1	177.4	Picoxystrobin 22.5	QoI	11	DuPont
Evito®480SC	1.0	59.1	Fluoxastrobin 40.3	QoI	11	Arysta
Headline®2.08EC	3.1	177.4	Pyraclostrobin 23.6	QoI	11	BASF
Priaxor®	2.1	118.3	Fluxapyroxad 14.33 + Pyraclostrobin 28.58	Carboxamides + QoI	7,11	BASF
Quadris®	3.1	177.4	Azoxystrobin 22.9	QoI	11	Syngenta
Sercadis®	1.4	79.8	Fluxapyroxad 26.55	Carboxamides	7	BASF
Stratego® YLD	2.1	118.3	Prothioconazole 10.8 + Trifloxystrobin 32.3	DMI + QoI	3,11	Bayer

Table 1: List of fungicides tested in *in vitro* on major soybean pathogens.[1]Dilutions were based on [2]labeled spray rates mixed in 56.7 liter of water. SC = Suspension concentrate; EC = Emulsifiable concentrate; QoI fungicides = Quinone outside inhibitors; DMI fungicides = DeMethylation inhibitors; *Fungicide Resistance Action Committee.

Under aseptic conditions, in a pre-disinfected NuAire class II type B2 biological safety cabinet, syringed 3.1-ml of product individually from the containers of Aproach, Headline EC, and Quadris, and transferred separately to conical flasks containing 1-liter sterilized deionized water (SDW). Each of the dilution was well mixed by stirring on thermolyne magnetic stir plate for two minutes. Similarly, the stock solutions of other fungicides were prepared (syringed 1-ml of Evito, 2.1-ml of Priaxor, 1.4-ml of Sercadis and 2.1-ml of Stratego YLD and were transferred separately in 1-liter SDW).

Plating and incubation

The fungal isolates were subcultured on a 5-mm thick PDA in 9-cm disposable petri dishes. In a modified dual culture plug technique [27], a single 6-mm culture plug taken from the edges of actively growing cultures using sterile cork borer, and placed inverted on one end inside periphery of PDA dishes and on the opposite end 6-mm sterilized blotter disc (Anchor Paper Co. Minnesota) was placed and soon after, 50-µl fungicide solution was transferred on the disc using microliter pipette (Rainin instrument Co., Inc, California) under aseptic conditions. To test against *S. glycines*, 50-µl spore suspension (1×10^8 spores/ml) was spread on to V8-plates using disposable Lazy-L-spreaders and transferred 50-µl fungicide solution on blotter disc

placed in the center. Each lidded plate was sealed with parafilm (Bemis Flexible Packaging, 2301 Industrial Drive Neenah, WI 54956) against moisture and air contamination. Sealed plates were transferred to pre-disinfected clear square plastic container (interior dimensions 25.4×17.8×7.6 cm *Pioneer Plastics*, *Inc. KY* 42409) and were incubated at 23 ± 1˚C in 12h fluorescent light at visible light intensity of 0.42 w/m^2 (measured using PMA2100, Solar light company, Inc. 100 East Glenside Ave, Glenside, Pennsylvania) for 12 days. There were four replications for each of the pathogen and fungicide combination and control plates.

Assessment of *in vitro* effects of fungicides on growth of pathogens

Radial growth rates (mm/day) of pathogens in presence and absence of fungicides was measured from the edge of the culture plug. Also documented photographs of culture plates with or without fungicide discs. Percent reduction in radial growth of pathogens in presence of fungicides compared with control was calculated following the formula given below,

Reduction (%) in radial growth of pathogen = (GAF– GPF) ÷ GAF × 100.

Where, GAF= Radial growth or radius of pathogen in the absence of fungicide and

GPF= Radial growth or radius of pathogen in the presence of fungicide (Figure 1A).

Assessment of *in vitro* sensitivity of pathogens to fungicides

Inhibition zone formation is an indication of fungicidal or lethal effect of fungicides on growth and reproduction of pathogens. In other words, sensitivity of pathogens to fungicides. To assess the sensitivity, inhibition zone size (mm) between growth end of pathogen and edge of fungicide disc or inhibition zone size diameter (mm) around fungicide disc (only in *S. glycines*) was measured (Figure 1B). Also documented photographs of visual inhibition zone formed between pathogens and fungicide disc.

Assessment of *in vitro* persistence of pathogens' tolerance to fungicides

Fungistatic effect (Figure 1C) is inhibiting growth of fungi without destroying them or inhibiting the growth of fungi temporarily [28-30]. Fungistatic effect is seen because of fungicides diffusion on synthetic medium. The Fungistatic effects of fungicides was measured (mm) at least in three pathogens, also documented photographs of Fungistatic effects.

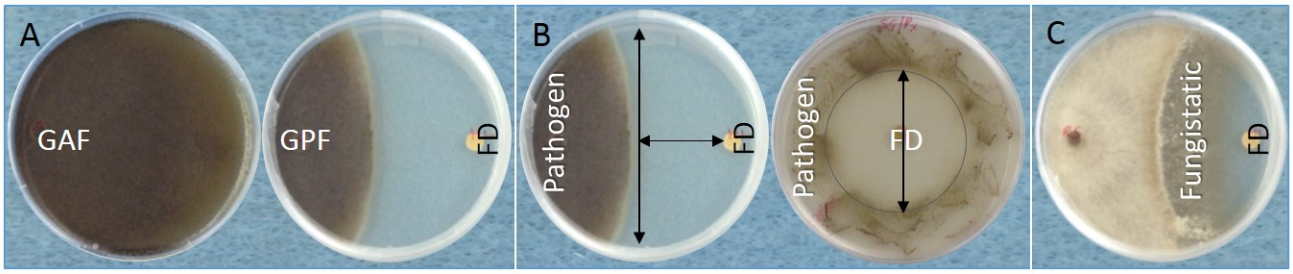

Figure 1: Measurement of (A) percent reduction in radial growth of pathogen = (GAF–GPF) ÷ GAF × 100. Where, GAF is radial growth of pathogen in the absence of fungicide disc (FD) and GPF is radial growth of pathogen in the presence of FD, (B) inhibition zone between growth end of pathogen and edge of FD or around FD and (C) Fungistatic effect is inhibiting growth of fungi temporarily without destroying them.

Assessment of area under colony growth

The area under colony growth of fungi was measured using individual digital images at Image Analysis Facility, Office of Biotechnology, Iowa State University, Ames, Iowa. Individual digital pictures of fungi grown on 9-cm PDA Petri dish, either with fungicide disc (FD) placed on opposite end of test fungus or without FD were taken using Leica V-Lux 30. Using Petri dish size as parameter, the ImageJ software [31] was calibrated for each set of Petri dishes prior to measurement of the colony growth using the Set Scale function of ImageJ (v. 1.45s). All measurements were done in square cm. To quantify the area occupied by the fungus, each colony area on the color images was outlined. The color images were then split into individual red, green, and blue channels using the Split Channels function. The resulting image with the best contrast between the background and the outline was interactively thresholded to isolate the outlines using the Threshold function. The outlines were then filled and individually measured using the Fill Holes and Measure functions, respectively. The resulting measurements were transferred to a spreadsheet for further analysis.

Data analysis

Mean radial growth of pathogens in presence and absence of fungicides, reduction percent in growth of pathogens compared with control, sensitivity of pathogens to fungicides and fungicide tolerance of pathogens was analyzed using PROC ANOVA in SAS 9.4. (SAS, LLC, Cray, NY). Fisher's least significant difference was used to detect the significant differences among the means (P = 0.05).

Results

In vitro effects of fungicides on growth of pathogens

In control plates, *P. irregulare* and *S. sclerotiorum* reached the opposite end of the plate within 4 days, followed by *M. phaseolina*, and *R. solani* in 8 days and *C. truncatum* in 20 days but *F. virguliforme*

didn't reach periphery with an incubation of 24 days. In an *in vitro* assay, we investigated whether pathogens can grow efficiently on PDA in presence of fungicide disc placed on the opposite end of culture plug. All the six fungicides significantly (P<0.05) reduced radial growth of *C. truncatum* compared with control in 12 days after incubation (DAI). However, reduction (%) in growth varied depending on fungicide on the opposite end (Figures 2A and 2B). Reduction in radial growth of *C. truncatum* was significantly (P<0.05) highest in Headline EC and Stratego YLD (62.7%) followed by Priaxor and Quadris (55.2%), Aproach (50.7%), Evito (49.6%) and only 2.9% in Sercadis (Figure 2A). Reduction in radial growth of *F. virguliforme* was significantly highest in Stratego YLD (50%) followed by 44% in Aproach and Priaxor, 22% in Headline EC and 6% in Quadris (Figure 2A). However, Evito and Sercadis were not different from the control plates (Figure 2A). In *M. phaseolina*, significantly (P<0.05) highest reduction in radial growth was observed in Stratego YLD (49%) followed by Priaxor (35%) and Headline EC (24%) compared with control plates and other four fungicides (Figure 2A). Compared with control plates, plates with Priaxor significantly (P<0.05) reduced the radial growth of P. irregulare (26%) followed by Headline EC (24%), Quadris (21%), Aproach (21%), Evito (14%) and Stratego YLD (14%) in 4DAI (data not shown in Figure 2A).

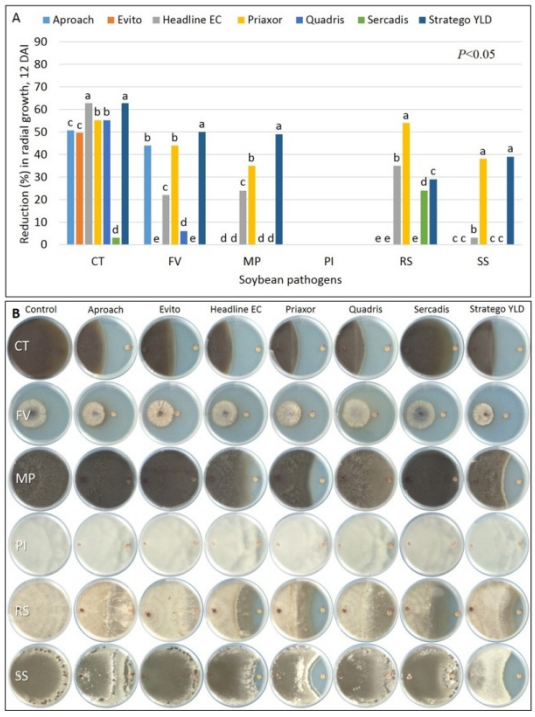

Figure 2: (A) Mean percent reduction in radial growth of *Colletotrichum truncatum* (CT), *Fusarium virguliforme* (FV), *Macrophomina phaseolina* (MP), *Pythium irregulare* (PI), *Rhizoctonia solani* (RS), and *Sclerotinia sclerotiorum* (SS) in presence of fungicide disc compared with control on PDA plates in 12 days after incubation and (B) colony growth, sensitivity and fungistatic effects on individual pathogens in presence of fungicide disc compared with control. Means in individual pathogens followed by the same letter(s) are not significantly different from each other at 5% level of significance (P<0.05).

However, none of the fungicides had any effect on *P. irregulare* compared with control in extended incubation of 12 days (Figures 2A and 2B). In *R. solani*, significant reduction was observed in plates with Priaxor (54%), followed by Headline EC (35%), Stratego YLD (29%) and Sercadis (24%). However, Aproach, Evito, and Quadris fungicides had no impact on growth of *R. solani* compared with control plates (Figures 2A and 2B). In *S. sclerotiorum*, Stratego YLD showed 39% reduction in radial growth, followed by Priaxor (38%) and Headline EC (3%). Apart from reducing the mycelial growth of *S. sclerotiorum*, Stratego YLD, Priaxor and Headline EC also significantly (P<0.05) reduced sclerotia production compared with other fungicides treatments and control (Figure 2B). However, Aproach, Evito, Quadris, and Sercadis did not show any reduction in growth and reproduction of *S. sclerotiorum* (Figures 2A and 2B).

In vitro sensitivity of pathogens to fungicides

Inhibition zone (IZ) formation is an indication of sensitivity of pathogens to fungicides in their growth and reproduction. All the seven fungicides significantly (P<0.05) formed IZ between growth end of *C. truncatum* and fungicide disc except Sercadis (Figure 3A).

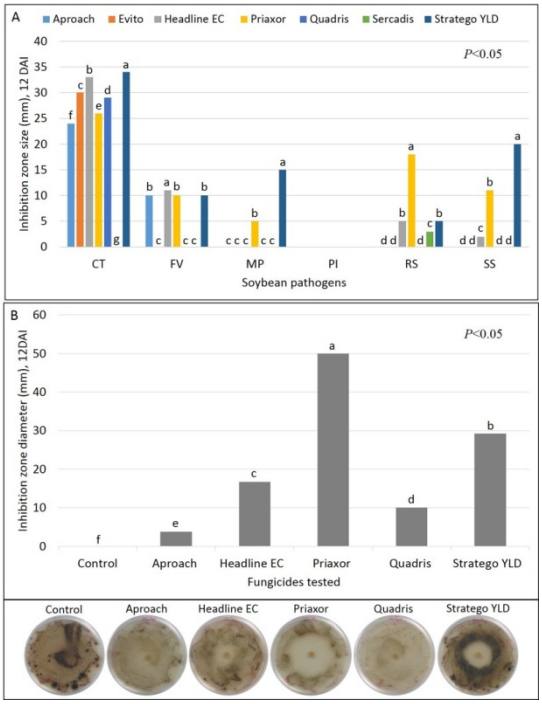

Figure 3: (A) Mean growth inhibition zone size (mm) between the growth ends of pathogens and edge of fungicide disc on PDA, and (B) Mean growth inhibition zone diameter size (mm) of Septoria glycines around fungicide disc placed in the center of PDA plates in 12 days after incubation. Pathogens: *C. truncatum* (CT), *F. virguliforme* (FV), *M. phaseolina* (MP), *P. irregulare* (PI), *R. solani* (RS), *S. glycines* (SG) and *S. sclerotiorum* (SS). Means in individual pathogens followed by the same letter(s) are not significantly different from each other at 5% level of significance (P<0.05).

Highest IZ was observed in plates with Stratego YLD (34 mm), followed by Headline EC (33 mm), Evito (30 mm), Quadris (29 mm), Priaxor (26 mm), and Aproach (24 mm). In *F. virguliforme*, Headline

EC had the highest IZ (11 mm) compared with 10 mm in Aproach, Priaxor, and Stratego YLD. However, there was no IZ observed in plates with Evito, Quadris and Sercadis (Figure 3A). Stratego YLD showed significant (P<0.05) IZ (15 mm) in *M. phaseolina* followed by Priaxor (5 mm), and none of the other fungicides tested showed any IZ (Figure 3A). *P. irregulare*, did not show sensitivity to any of the fungicides tested in 12 DAI (Figure 3A), but in 4DAI, in plates with Aproach and Stratego YLD the IZ was 10-mm, followed Priaxor with 8 mm, and Evito and Headline EC with 5 mm (data not shown). *R. solani* plated with Priaxor showed significantly (P<0.05) highest IZ (18 mm) followed by 5 mm in Headline EC and Stratego YLD, and 3 mm in Sercadis (Figure 3A). In *S. sclerotiorum*, plates with Stratego YLD showed significantly highest IZ (20 mm) followed Priaxor (11 mm) and Headline EC (2 mm) and other four fungicides did not form any IZ against *S. sclerotiorum* (Figure 3A).

In case of *S. glycines*, plating was different from six other pathogens tested. Significantly highest IZ diameter (50 mm) was observed in plates with Priaxor followed by Stratego YLD (29 mm), Headline EC (17 mm), Quadris (10 mm) and Aproach (4 mm) compared with control (Figure 3B).

In vitro persistence of fungicide tolerance in pathogens

Fungistatic effect is inhibiting growth of fungi without destroying them or inhibiting the growth of fungi temporarily [28-30]. All fungicides except Sercadis showed Fungistatic effect. With an increased incubation period, odds of observing Fungistatic effect are more if the product has the ability to slower the growth rate of pathogens. This happens because of fungicides diffusion on synthetic medium. During diffusion process fungicide moves down the concentration gradient, that means, concentration of fungicide is higher where the disc was placed, away from the disc, concentration reduces due to diffusion. Fungistatic effect was observed only in *C. truncatum*, *M. phaseolina* and *R. solani* starting 8 DAI. The Fungistatic effect on *C. truncatum* was significantly higher (8 mm) in plates with Headline EC and Aproach followed by Evito, Priaxor, and Stratego YLD each with 5 mm in 8 DAI. While in 12 DAI, Fungistatic effect on *C. truncatum* was significantly higher (14 mm) in plates with Aproach, Evito, and Quadris, followed by Priaxor with 11 mm, and Headline EC and Stratego YLD with 8 mm (Figures 4A and 4B).

The Fungistatic effect on *M. phaseolina* was observed within 4 DAI. Significantly highest (27 mm) Fungistatic effect was observed in plates with Quadris followed by Headline EC (25 mm), Priaxor and Stratego YLD (16 mm), Aproach (14 mm), Sercadis (14 mm), and Evito (8 mm) in 4DAI. Whereas, in 8 DAI, significant Fungistatic effect size of 27 mm was observed in Aproach, Headline EC, Quadris, Sercadis, and Stratego YLD compared with Priaxor (16 mm) and Evito (14 mm). In 12 DAI, significantly highest (41 mm) Fungistatic effect was observed in Quadris, compared with Headline EC (33 mm), Aproach, Evito, and Stratego YLD with 27 mm. and Priaxor with 16 mm. However, Fungistatic effect in Sercadis reduced from 27 mm in 8DAI to zero in 12DAI (Figures 4A and 4B).

Similar to *M. phaseolina*, the Fungistatic effect on *R. solani* was also observed within 4 DAI. Significantly highest (14 mm) Fungistatic effect was observed in plates with Sercadis followed by Evito (11 mm), whereas in plates with Aproach, Headline EC, Priaxor, and Quadris with 8 mm and Stratego YLD did not show Fungistatic effect in 4 DAI. In 8 DAI, significantly highest (35 mm) Fungistatic size was observed in Quadris, followed by plates with Aproach, Evito and Sercadis 27 mm, Headline EC (22 mm) Priaxor (19 mm) and Stratego YLD (14 mm). Whereas, in 12 DAI, Fungistatic size in Aproach, Headline EC, Quadris and Sercadis was 46 mm, followed by Evito (41 mm), and Priaxor and Stratego YLD with 27 mm each. (Figures 4A and 4B).

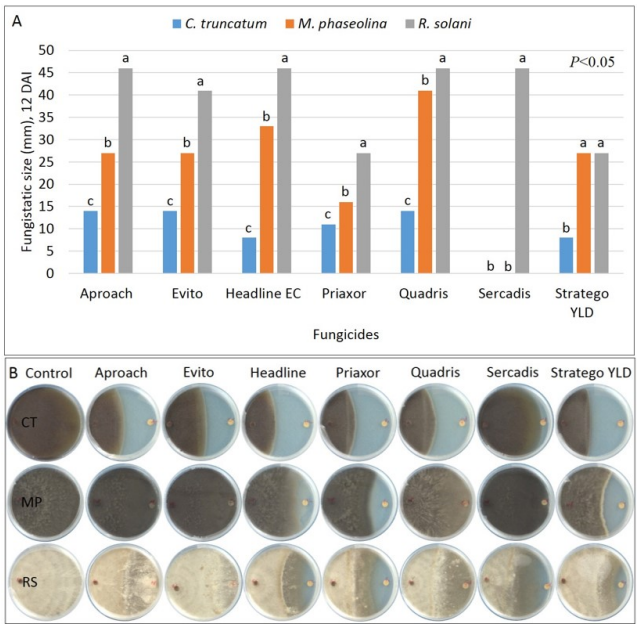

Figure 4: (A) Mean fungistatic effect size (mm) of *C. truncatum*, *M. phaseolina* and *R. solani* and (B) corresponding images showing fungistatic effect on PDA plates sown with fungicide disc on the opposite end compared with their controls. Means of fungi followed by the same letter(s) in each fungicide are not significantly different from each other at 5% level of significance (P<0.05).

	Area under colony growth (cm^2)						
Treatments	**CT**	**FV**	**MP**	**PI**	**RS**	**SG**	**SS**
Control	59.5a	17.1a	63.6a	63.6a	63.6a	63.6a	63.6a
Aproach®	32.1d	12.1g	63.6a	63.6a	63.6a	63.6a	63.6a
Evito®480SC	39.8c	15.8d	63.6a	63.6a	63.6a	-*	63.6a
Headline®2.08EC	26.4h	14.1e	60.3b	63.6a	54.3c	58.0b	57.2c
Priaxor®	29.2g	12.9f	55.7c	63.6a	44.8e	42.4d	53.4d
Quadris®	31.1e	16.1c	63.6a	63.6a	63.6a	63.6a	63.6a
Sercadis®	54.1b	16.9b	63.6a	63.6a	54.8b	-	61.9b
Stratego® YLD	30.0f	10.0h	45.2d	63.6a	52.4d	55.3c	44.8e

Table 2: [1]Mean area under colony growth (cm^2) of *Colletotrichum truncatum* (CT), *Fusarium virguliforme* (FV), *Macrophomina phaseolina* (MP), *Pythium irregulare* (PI), *Rhizoctonia solani* (RS), *Septoria glycines* (SG) and *Sclerotinia sclerotiorum* (SS) in presence and absence (control) of fungicide disc on PDA plates in 12 days after incubation.[1]Results are average of four plates. Means within column followed by the same letter(s) are not significantly different from each other at 5% level of significance (P<0.05). *Not tested.

Area under colony growth

The mean area (cm2) under colony growth (AUCG) of *C. truncatum* and *F. virguliforme* was significantly (P<0.05) less in plates with fungicide discs compared with control (Table 2). The AUCG of *M. phaseolina* was significantly less in plates with Stratego YLD, Priaxor and Headline EC compared with control and other fungicides. Similarly, the AUCG of *R. solani*, *S. glycines* and *S. sclerotiorum* was significantly (P<0.05) less in Priaxor, Headline, Stratego YLD and Sercadis compared with control and other fungicides (Table 2).

Discussion

Fungicides toxic to fungi affect in several ways. The mycelium may cease growing, change metabolic processes or be killed, spores may fail to germinate or be killed [31]. Fungicides tested in this study were foliar applied to control various diseases and or for plant health benefits in soybean. Results of quadruplicate for each combination of pathogen and fungicide have significantly (P<0.05) reduced radial growths of majority of pathogens tested compared with controls. Headline, Priaxor and Stratego YLD fungicides were effective against all the pathogens tested except *P. irregulare*. Overall, Aproach was effective against *C. truncatum*, *F. virguliforme* and *S. glycines*, Evito against *C. truncatum*, Quadris against *C. truncatum*, *F. virguliforme* and *S. glycines*, and Sercadis *C. truncatum* and *R. solani*. However, degree of effectiveness of fungicides tested against seven pathogens varied based on their growth rates and sensitivity to fungicides. Another parameter used to compare the effectiveness of fungicides was area under colony growth on ImageJ software [32]. Results of this study indicate that, the higher the area under colony growth (AUCG), lower the effect of fungicide against a fungi (Table 2). It is important to note that AUCG doesn't exclude fungistatic effect showed in Figures 4A and 4B.

After reviewing product labels, we tend to believe that *in vitro* test results either complement the assertion on the label or differ and provide additional information to the label (pending, either seed treatment or foliar field tests). According to DuPont, Aproach® (*Picoxystrobin*) is effective against *S. sclerotiorum*, frogeye leaf spot (*Cercospora sojina*), brown spot (*S. glycines*) and Asian soybean rust (*Puccinia pachyrhizi*) in soybeans. Out of these, *in vitro* results showed Aproach was effective against and *S. glycines* as labeled but also effective against *C. truncatum* and *F. virguliforme* (not labeled). Although, Aproach was not the highest radial growth reducer of *C. truncatum* compared with Headline EC, Priaxor, Quadris, and Stratego YLD but was significantly higher than Sercadis (Figures 2A and 2B). Similarly, Aproach has effectively reduced the radial growth of *F. virguliforme* on par with Priaxor, and significantly lower than Stratego YLD but higher than other fungicides (Figures 2A and 2B). Whereas, in *S. glycines*, Aproach showed lowest inhibition diameter compared with Priaxor, Stratego YLD and Headline EC. Also, Aproach was ineffective on *P. irregulare*, *M. phaseolina*, *R. solani* (not listed on the label) and *S. sclerotiorum* (listed on the label). Similar *in vitro* observation has been reported about Aproach on *S. sclerotiorum* compared with Endura fungicide [33]. As per the label, Aproach should have suppressed growth of *S. sclerotiorum* instead it was on par with control plates both in terms of mycelial growth and also reproduction of sclerotia (Figure 2B). Chances are the product may be effective against ascospores than suppressing sclerotia production per se or the product may show better results in field conditions (either seed treatment or foliar applied) compared with *in vitro* tests. We do not intend to extrapolate ineffectiveness of Aproach against *S. sclerotiorum* based on the *in vitro* results as was suggested by De Clercq [34].

According to Arysta Lifescience, Evito® (fluoxastrobin) with its advanced Strobilurin chemistry, delivers outstanding control of alternaria leaf spot (*Alternaria spp.*), anthracnose (*Colletotrichum spp.*), brown spot (*S. glycines*), cercospora blight (*Cercospora kikuchii*), frogeye leaf spot (*C. sojina*), pod and stem blight (*Diaporthe phaseolorum*), rhizoctonia aerial blight (*R. solani*) and rust (*P. pachyrhizi*) in soybean. Out of these, *in vitro* tests of Evito were against labeled *C. truncatum*, *R. solani*, and not labeled *F. virguliforme*, *M. phaseolina*, *P. irregulare*, and *S. sclerotiorum*. Evito was as effective as Aproach on *C. truncatum*, but it did not show any effect on other pathogens tested including *R. solani* (Figures 2A and 2B). Also, field tests by Giesler [35] showed, no significant effect on brown spot (*S. glycines*) severity (11-47 days after spray) and yield.

Three BASF Corporation products Headline, Priaxor, and Sercadis were tested. According to BASF, Headline® (pyraclostrobin) applied in-furrow on corn and soybean, helps control soil borne R. solani while providing plant health benefits, including healthier, more vigorous roots. In addition, it helps improve seedling health and allows for more rapid and uniform emergence even under cold and wet conditions. Plus the EC formulation can be tank-mixed with a liquid fertilizer for easy application. In *in vitro* tests, Headline has significantly (P<0.05) reduced the radial growths of *C. truncatum*, *F. virguliforme*, *M. phaseolina*, *S. sclerotiorum* (not labeled) and *R. solani* (labeled) but not *P. irregulare* (Figures 2A and 2B). *In vitro* results showed *C. truncatum*, *F. virguliforme*, *R. solani*, *S. sclerotiorum* and *S. glycines* significant sensitivity to Headline. Interestingly, Headline, either solo or in combination with other fungicides and insecticides significantly (P<0.05) suppressed brown spot (*S. glycines*), and frogeye leaf spot (*C. sojina*) across 11 seasons, with an average yield advantage of 5 bu/ac or 0.26 mt/ha (range 2 to 8 bu/ac or 0.10 to 0.41 mt/ha), even under low diseases pressure [36]. Although, no significant advantage of Headline (solo or combination) in plots with sudden death syndrome (*F. virguliforme*) and white mold (*S. sclerotiorum*), but significant (P<0.05) yield increase was observed over unsprayed controls indicating plant health benefits of spray [36].

Priaxor® (fluxapyroxad + pyraclostrobin), as a foliar spray is effective against alternaria leaf spot (*Alternaria* spp.), anthracnose (*C. truncatum*), Asian soybean rust* (*P. pachyrhizi* *not registered for use in California), brown spot (*S. glycines*), Cercospora blight (*C. kikuchii*), frogeye leaf spot (*C. sojina*), pod and stem blight (*D. phaseolorum*), Rhizoctonia aerial blight (*R. solani*), suppression only white mold (*S. Sclerotiorum*) and southern blight (*Sclerotium rolfsii*) as per the label. Out of this list, *in vitro* test was conducted against labeled *C. truncatum*, *R. solani*, *S. glycines*, and *S. sclerotiorum*, and not labeled *F. virguliforme*, *M. phaseolina*, and *P. irregulare*. Priaxor was significantly effective in reducing the radial growths of labeled and not labeled pathogens (except *P. irregulare*) compared with control (Figures 2A and 2B). Priaxor was also significantly effective in field sprays against white mold and yields [37,38] and significant effect on white mold but not on yields [39].

A third BASF product, Sercadis® (fluxapyroxad) fungicide provides both preventive and post infection sheath blight of rice (*R. solani*) control with long-lasting residual. Irrespective of crop, Sercadis significantly reduced radial growth of *C. truncatum* (not labelled) and *R. solani* (labelled) compared with control (Figures 2A and 2B). We do not wish to speculate if Sercadis could be effective against these two soybean pathogens in field tests.

According to Bayer CropScience, Stratego® YLD (prothioconazole + trifloxystrobin) controls alternaria leaf spot (*Alternaria* spp.), anthracnose (*C. truncatum*), Asian soybean rust (*P. pachyrhizi*), brown spot (*S. glycines*), cercospora blight (*C. kikuchii*), frogeye leaf spot (*C. sojina*), pod and stem blight (*D. phaseolorum*), powdery mildew (*Microsphaera diffusa*), Rhizoctonia aerial blight (*R. solani*). Out of this list, *in vitro* efficacy tests were conducted against *C. truncatum*, *R. solani*, *S. glycines* (listed on label), *F. virguliforme*, *M. phaseolina*, and *S. sclerotiorum* (not listed on label). *In vitro* results showed significant reduction in growth of both labeled and not labeled pathogens except *P. irregulare* compared with control (Figures 2A and 2B). Foliar spray of Stratego YLD, did not show significant effect on white mold and yields [37], and on sudden death syndrome and white mold and yield [8]. Also, field tests by Giesler [35] indicated, no significant effect on brown spot severity (11-47 days after spray) and yield. In 2015 seed treatment tests with these products, some of the fungicides showed significant increase in stand count, suppression of *F. virguliforme* and *R. solani* compared with control [40].

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Disclaimer

Products tested in this study do not imply endorsement of one company over another, nor was discrimination intended against any similar products. BASF had no role in the data collection and analysis or the preparation of this article.

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