CHAPTER - I

ASSESSMENT OF FOLIAR FUNGICIDES AGAINST SOME MAJOR SOIL-BORNE PATHOGENS OF SOYBEAN

Abstract

Seven foliar fungicides were assessed 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 five fungicides against Septoria glycines (SG). Under aseptic conditions, a single 6-mm culture plug of actively growing individual fungi were 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 was 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-d incubation in 12-h photoperiod, assessed in vitro the effects of fungicides on growth, sensitivity and tolerance in pathogens. All the fungicides except Sercadis, significantly (P<0.05) reduced radial growth of CT, while Headline, Priaxor and Stratego YLD significantly reduced growth of FV, MP, RS and SS. The SG and CT showed significant (P<0.05) sensitivity to most of the fungicides. Whereas, FV, RS and SS showed significant sensitivity to Headline, Priaxor and Stratego YLD. The CT, MP and RS showed significant (P<0.05) persistence to all the fungicides, in other words is consider that as Fungistatic effect.

Keywords: Fungicides, Strobilurin; Triazole, Pyrazole-caroxamide, Fungistatic effect, Soybean pathogens.
INTRODUCTION

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 (Wrather and Koenning, 2006). However, these diseases do reduce photosynthetic activity in infected leaves by reducing green leaf area and affecting photosynthesis in the asymptomatic area of diseases infection (Bassanezi *et al*., 2001; Shtienberg, 1992). 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% (Wrather and Koenning, 2009). According to USDA-NASS (2013) fungicides use in soybean has gone up from <1% of the soybean planted acreage in 20 program states to 11% of soybean planted acres in 2012 and it remain unchanged 2015 crop year in 19 program states (USDA-NASS, 2016).

Several seed treatment products (chemical and biological) were tested against *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, *Phytophthora sojae* (Bradley, 2008), and against *Sclerotinia sclerotiorum* both in vitro and field (Mueller *et al*., 1999). Also, some specific active ingredients like azoxystrobin (Bartlett *et al*., 2002; Ramirez, 2004; Broders *et al*., 2007), pyraclostrobin (Ellis *et al*., 2011), trifloxystrobin (Chala *et al*., 2003), thiophanate-methyl (Yoshida *et al*., 2008), prothioconazole (Paul *et al*., 2008), and fludioxonil (Hewitt, 1998; Broders *et al*., 2007; Ellis *et al*., 2011) 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 current approach is similar to Powelson and Inglis (1999) to identify a 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 (Conner, 1983), food poisoning technique (Adams and Wong, 1991), agar-well diffusion technique (Magaldi *et al*., 2001), poison plate and spore germination tests (Corden and Young, 1962; Anahosur *et al*., 1977; Everett *et al*., 2005). In the current study, a modified dual culture plug technique of Rahman *et al*., (2009) was adopted to test foliar fungicides against some major soil-borne pathogens of soybean. Objectives of the current study were to test *in vitro* effects of fungicides on growth, sensitivity and persistence of pathogens’ tolerance to fungicides or 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 (Plate-1). 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 pre-sterilized polystyrene disposable Lazy-L-spreaders (Research Products International Corp.) to
loosen the conidia. Pipetted 50-µl to a fresh V8 plate and spread with a disposable lazy-L-spreader, incubated at 23±1°C in 12 h light on 12 h off cycle for a week.

PLATE-1: Colonies, and fruiting bodies of (Fig. a) *Colletotrichum truncatum*, (Fig. b) *Fusarium virguliforme*, (Fig. c) *Macrophomina phaseolina*, (Fig. d) *Pythium irregulare*, (Fig. e) *Rhizoctonia solani*, (Fig. f) *Sclerotinia sclerotiorum* and (Fig. g) *Septoria glycines*.

Fungicides stock solution preparation.

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

**Table-1:** List of foliar fungicides tested *in vitro* and *in vivo* against some major soil borne pathogens and diseases of soybean.

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Dilutions used (ml/L)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Labeled rates ml/ha&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Active Ingredient (%)</th>
<th>Group</th>
<th>FRAC Code*</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aproach 250SC</td>
<td>3.1</td>
<td>438.2</td>
<td>Picoxystrobin 22.5</td>
<td>QoI</td>
<td>11</td>
<td>DuPont</td>
</tr>
<tr>
<td>Evito 480SC</td>
<td>1.0</td>
<td>146</td>
<td>Fluoxastrobin 40.3</td>
<td>QoI</td>
<td>11</td>
<td>Arysta LifeSciences</td>
</tr>
<tr>
<td>Headline 250EC</td>
<td>3.1</td>
<td>438.2</td>
<td>Pyraclostrobin 23.6</td>
<td>QoI</td>
<td>11</td>
<td>BASF</td>
</tr>
<tr>
<td>Priaxor 500SC</td>
<td>2.1</td>
<td>292.2</td>
<td>Fluxapyroxad 14.33 +</td>
<td>Carboxamides + 7, 11</td>
<td>BASF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pyraclostrobin 28.58</td>
<td>QoI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadris 250FL</td>
<td>3.1</td>
<td>438.2</td>
<td>Azoxystrobin 22.9</td>
<td>QoI</td>
<td>11</td>
<td>Syngenta</td>
</tr>
<tr>
<td>Sercadis 300SC</td>
<td>1.4</td>
<td>197.1</td>
<td>Fluxapyroxad 26.55</td>
<td>Carboxamides 7</td>
<td>BASF</td>
<td></td>
</tr>
<tr>
<td>Stratego YLD</td>
<td>2.1</td>
<td>292.2</td>
<td>Prothioconazole 10.8 +</td>
<td>DMI +</td>
<td>3, 11</td>
<td>Bayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trifloxystrobin 32.3</td>
<td>QoI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Dilutions were based on <sup>2</sup>labeled spray rates mixed in 140 liter of water. SC = Suspension concentrate; EC = Emulsifiable concentrate; FL = Flowable; QoI fungicides = Quinone outside inhibitors; DMI fungicides = DeMethylation inhibitors; *Fungicide Resistance Action Committee.

**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 (Rahman *et al.*, 2009), 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 SG, 50-μl spore suspension \((1 \times 10^8 \text{ spores/ml})\) 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 \(\times\) 17.8 \(\times\) 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/m2 measured using PMA2100 (Solar light company, Inc. 100 East Glenside Ave, Glenside, Pennsylvania) for 12d.

**Effect of fungicides on growth of pathogens**

Radial growth rates (mm/day) of pathogens in the 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.

Reduction (%) in growth of pathogens in the presence of fungicides compared with control was calculated using the formula given in Plate-2; Fig. a.

![Plate-2](image)

**PLATE-2:** Measurement of (Fig. a) percent reduction in radial growth of pathogen = \((\text{GAF} – \text{GPF}) ÷ \text{GAF} \times 100\). Where, GAF and GPF are radial growth of pathogen (mm) in the absence and presence of fungicide disc (FD) respectively, (Fig. b) inhibition zone between growth end of pathogen and edge of FD or around FD and (Fig. c) Fungistatic effect is inhibiting growth of fungi without destroying or inhibiting the growth of fungus temporarily.
Sensitivity of pathogens to fungicides

Inhibition zone (IZ) 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. The IZ (mm) between growth end of pathogen and edge of fungicide disc or diameter around fungicide disc only in *S. glycines* was measured (Plate-2; Fig. b).

Persistence of pathogens’ tolerance to fungicides

Fungistatic effect is inhibiting the growth of fungi without destroying them or inhibiting the growth of fungi temporarily (Mostafa *et al*., 2011; Al-Rahmah *et al*., 2013; Shrestha *et al*., 2015). Fungistatic effect is seen because of fungicides diffusion on synthetic medium and was measured (mm) as showed in Plate-2; Fig. c.

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 plate size as parameter, the ImageJ software (Abramoff, *et al*., 2004) 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). 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 (cm²) were transferred to a spreadsheet for further analysis.
Data analysis

Mean radial growth, % reduction in growth, sensitivity 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

Effects of fungicides on growth of pathogens

In control plates, PI and SS reached the opposite end of the plate within 4 days, followed by MP, and RS in 8 days and CT in 20 days but FV didn’t reach periphery with an incubation of 24 days. In an *in vitro* assay, investigated whether pathogens can grow efficiently on PDA in presence of fungicide disc placed on the opposite end of culture plug. All the seven fungicides significantly ($P < 0.05$) reduced radial growth of CT compared with control in 12 days after incubation (DAI). However, reduction (%) in growth varied depending on fungicide on the opposite end (Plates-9 and 10). Reduction in radial growth of CT was significantly ($P < 0.05$) highest in Headline and Stratego YLD (62.7%) followed by Priaxor and Quadris (55.2%), Aproach (50.7%), Evito (49.6%) and only 2.9% in Sercadis (Plate-9). Reduction in radial growth of FV was significantly highest in Stratego YLD (50%) followed by 44% in Aproach and Priaxor, 22% in Headline and 6% in Quadris (Plate-3). However, Evito and Sercadis were not different from the control plates (Plates-3 and 4). Significantly ($P < 0.05$) highest reduction in radial growth of MP was observed in Stratego YLD (49%) followed by Priaxor (35%) and Headline (24%) compared with control plates and other four fungicides (Plate-3). Compared with control plates, Priaxor significantly ($P < 0.05$) reduced the radial growth of PI (26%) followed by Headline (24%), Quadris (21%), Aproach (21%), Evito (14%) and Stratego YLD
(14%) in 4DAI (data not shown in Plate-3). However, none of the fungicides had any effect on *P. irregulare* compared with control in 12 days extended incubation (Plates-3 and 4).

**PLATE-3:** Mean reduction (%) in radial growth of pathogens (CT= *C. truncatum*, FV= *F. virguliforme*, MP= *M. phaseolina*, PI= *P. irregulare*, RS= *R. solani*, and SS= *S. sclerotiorum*) in presence of fungicide disc on PDA compared with control, 12 days after incubation (DAI). 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).

In RS, significant reduction was observed in plates with Priaxor (54%), followed by Headline (35%), Stratego YLD (29%) and Sercadis (24%). However, Aproach, Evito, and Quadris fungicides had no impact on growth of RS compared with control plates (Plate-3). In SS, Stratego YLD showed 39% reduction in radial growth, followed by Priaxor (38%) and Headline (3%). Apart from reducing the mycelial growth of SS, Stratego YLD, Priaxor and Headline also significantly (*P*<0.05) reduced sclerotia production compared with other fungicides treatments and control (Plate-3). However, Aproach, Evito, Quadris, and Sercadis did not show any reduction in growth and reproduction of SS (Plate-4).
**PLATE-4:** Growth, sensitivity and fungistatic effects on individual pathogens (CT= *C. truncatum*, FV= *F. virguliforme*, MP= *M. phaseolina*, PI= *P. irregulare*, RS= *R. solani*, and SS= *S. sclerotiorum*) in presence of fungicide disc compared with control on PDA plates, 12 days after incubation.

**Sensitivity of pathogens to fungicides**

All the seven fungicides significantly (*P*<0.05) formed IZ between growth end of CT and fungicide disc except Sercadis (Plate-5). The highest IZ was observed in Stratego YLD (34-mm), followed by Headline (33-mm), Evito (30-mm), Quadris (29-mm), Priaxor (26-mm), and Aproach (24-mm). In FV, Headline had the highest IZ (11-mm) compared with 10 mm in Aproach, Priaxor, and Stratego YLD. However, there was no IZ observed in Evito, Quadris and Sercadis (Plate-5). Stratego YLD showed significant (*P*<0.05) IZ (15-mm) in MP followed by Priaxor (5-mm), and none of the other fungicides tested showed any IZ (Plate-5). The PI did not show sensitivity to any of the fungicides tested in 12DAI (Plate-5), but in 4DAI, in plates
with Aproach and Stratego YLD the IZ was 10-mm, followed Priaxor with 8-mm, and Evito and Headline with 5-mm (data not shown). In RS plated with Priaxor showed significantly ($P<0.05$) highest IZ (18-mm) followed by 5-mm in Headline and Stratego YLD, and 3-mm in Sercadis (Plate-5). In SS, plates with Stratego YLD showed significantly highest IZ (20-mm) followed Priaxor (11-mm) and Headline (2-mm) and other four fungicides did not form any IZ against SS (Plate-5).

**PLATE-5:** Mean growth inhibition zone size (mm) between the growth end of pathogens (CT= *C. truncatum*, FV= *F. virguliforme*, MP= *M. phaseolina*, PI= *P. irregulare*, RS= *R. solani*, and SS= *S. sclerotiorum*) and edge of fungicide disc in PDA, 12 days after incubation (DAI). Means in individual pathogens followed by the same letter(s) are not significantly different from each other at 5% level of significance ($P<0.05$).

In case of SG, significantly highest IZ diameter (50-mm) was observed in plates with Priaxor followed by Stratego YLD (29-mm), Headline (17-mm), Quadris (10 mm), and Aproach (4-mm) compared with control (Plate-6).
PLATE-6: Mean growth inhibition zone diameter size (mm) of *Septoria glycines* around fungicide disc placed in the center of PDA plates, 12 days after incubation (DAI). Means followed by the same letter(s) are not significantly different from each other at 5% level of significance (*P*<0.05).

**Persistence of fungicide tolerance in pathogens**

Fungistatic effect is inhibiting growth of fungi without destroying them or inhibiting the growth of fungi temporarily (Mostafa *et al*., 2011; Al-Rahmah *et al*., 2013; Shrestha *et al*., 2015). 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. 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 CT, MP and RS starting 8DAI. The Fungistatic effect on CT was significantly higher (8 mm) in plates with Headline and Aproach followed by Evito, Priaxor, and Stratego YLD each with 5 mm in 8DAI. While in 12DAI, Fungistatic effect on CT was significantly higher (14 mm) in plates with Aproach, Evito, and Quadris, followed by Priaxor with 11 mm, and Headline and Stratego YLD with 8 mm (Plates-7 and 8).

PLATE-7: Mean Fungistatic effect size (mm) of *Colletotrichum truncatum*, *Macrophomina phaseolina* and *Rhizoctonia solani* in presence of fungicide disc compared with their controls, 12 days after incubation (DAI). Means in each of the fungicides followed by the same letter(s) are not significantly different from each other at 5% level of significance (*P*<0.05).

The Fungistatic effect on MP was observed within 4DAI. Significantly highest (27 mm) Fungistatic effect was observed in plates with Quadris followed by Headline (25 mm), Priaxor and Stratego YLD (16 mm), Aproach (14 mm), Sercadis (14 mm), and Evito (8 mm) in 4DAI. Whereas, in 8DAI, significant Fungistatic effect size of 27 mm was observed in Aproach, Headline, Quadris, Sercadis, and Stratego YLD compared with Priaxor (16 mm) and Evito (14 mm). In 12DAI, significantly highest (41 mm) Fungistatic effect was observed in Quadris, compared
with Headline (33 mm), Approach, 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 (Plates-7 and 8).

**PLATE-8**: Growth, sensitivity and fungistatic effects on pathogens (CT= *C. truncatum*, MP= *M. phaseolina*, and RS= *R. solani*), in presence of fungicide disc on the opposite end of fungus disc compared with control on PDA plates, 12 days after incubation.

Similar to MP, the Fungistatic effect on RS was also observed within 4DAI. Significantly highest (14 mm) Fungistatic effect was in plates with Sercadis followed by Evito (11 mm), whereas in plates with Approach, Headline, Priaxor, and Quadris with 8 mm and Stratego YLD did not show Fungistatic effect in 4DAI (Plates-13 and 14). In 8DAI, significantly highest (35 mm) Fungistatic size was observed in Quadris, followed by plates with Approach, Evito and Sercadis 27 mm, Headline (22 mm) Priaxor (19 mm) and Stratego YLD (14 mm). Whereas, in 12DAI, Fungistatic size in Approach, Headline, Quadris and Sercadis was 46 mm, followed by Evito (41), and Priaxor and Stratego YLD with 27 mm each (Plates-7 and 8).

**Area under colony growth**

The mean area (cm²) under colony growth (AUCG) of CT and FV was significantly ($P<0.05$) less in plates with fungicide discs compared with control (Table-2). The AUCG of
MP was significantly less in plates with Stratego YLD, Priaxor and Headline compared with control and other fungicides. Similarly, the AUCG of RS, SG and SS was significantly ($P<0.05$) less in Priaxor, Headline, Stratego YLD and Sercadis compared with control and other fungicides (Table-2).

**Table-2:** Mean area under colony growth ($\text{cm}^2$) of some major soil borne pathogens of soybean in presence and absence of fungicide disc on PDA plates in 12 days after incubation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CT</th>
<th>FV</th>
<th>MP</th>
<th>PI</th>
<th>RS</th>
<th>SG</th>
<th>SS</th>
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<tbody>
<tr>
<td>Control</td>
<td>59.5a</td>
<td>17.1a</td>
<td>63.6a</td>
<td>63.6a</td>
<td>63.6a</td>
<td>63.6a</td>
<td>63.6a</td>
</tr>
<tr>
<td>Aproach</td>
<td>32.1d</td>
<td>12.1g</td>
<td>63.6a</td>
<td>63.6a</td>
<td>63.6a</td>
<td>63.6a</td>
<td>63.6a</td>
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<tr>
<td>Evito</td>
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<td>15.8d</td>
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<td>63.6a</td>
<td>63.6a</td>
<td>-</td>
<td>63.6a</td>
</tr>
<tr>
<td>Headline</td>
<td>26.4h</td>
<td>14.1e</td>
<td>60.3b</td>
<td>63.6a</td>
<td>54.3c</td>
<td>58.0b</td>
<td>57.2c</td>
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<tr>
<td>Priaxor</td>
<td>29.2g</td>
<td>12.9f</td>
<td>55.7c</td>
<td>63.6a</td>
<td>44.8e</td>
<td>42.4d</td>
<td>53.4d</td>
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<td>Quadris</td>
<td>31.1e</td>
<td>16.1c</td>
<td>63.6a</td>
<td>63.6a</td>
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<td>63.6a</td>
<td>54.8b</td>
<td>-</td>
<td>61.9b</td>
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<td>Stratego YLD</td>
<td>30.0f</td>
<td>10.0h</td>
<td>45.2d</td>
<td>63.6a</td>
<td>52.4d</td>
<td>55.3c</td>
<td>44.8e</td>
</tr>
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</table>

1\text{Average of four plates. Colletotrichum truncatum (CT), Fusarium virguliforme (FV), Macrophomina phaseolina (MP), Pythium irregularare (PI), Rhizoctonia solani (RS), Septoria glycines (SG) and Sclerotinia sclerotiorum (SS) Means within column followed by the same letter(s) are not significantly different from each other at 5% level of significance ($P<0.05$).} 2\text{not tested.}

**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 (Neely, 1969). 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 were effective against all the pathogens tested except PI. Overall, Aproach and Quadris were effective against CT, FV and SG; Evito against CT, and Sercadis CT and RS. 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 AUCG using ImageJ software (Abramoff, 2004). Results of this study indicated that, the higher the AUCG, lower the effect of fungicide against a fungus (Table-2). It is important to note that AUCG doesn’t exclude fungistatic effect showed in Plates-7 and 8.

After reviewing product labels, in vitro test results either complement the assertion on the label or differ and provide additional information to the label. 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 SG as labeled but also effective against CT and FV (not labeled). Although, Aproach was not the highest radial growth reducer of CT compared with Headline, Priaxor, Quadris, and Stratego YLD but was significantly higher than Sercadis (Plates-3 and 4). Similarly, Aproach has effectively reduced the radial growth of FV on par with Priaxor, and significantly lower than Stratego YLD but higher than other fungicides (Plates-3 and 4). Whereas, in SG, Aproach showed lowest inhibition diameter compared with Priaxor, Stratego YLD and Headline and was ineffective on PI, MP, RS (not listed on the label) and SS (listed on the label). Similar in vitro observation has been reported about Aproach on SS compared with Endura fungicide (Navi et al., 2016). As per the label,
Aproach should have suppressed growth of SS, instead it was on par with control plates both in terms of mycelial growth and reproduction of sclerotia (Plate-4). 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. At this point it is not intend to extrapolate ineffectiveness of Aproach against SS based on the *in vitro* results as was suggested by De Clercq, (2005).

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 CT, RS, and not labeled FV, MP, PI, and SS. Evito was as effective as Aproach on CT, but it did not show any effect on other pathogens tested including RS (Plate-4). Also, field tests by Giesler (2012) 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 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 CT, FV, MP, SS (not labeled) and RS (labeled) but not PI (Plates-
3 and 4). In *in vitro* CT, FV, RS, SS and SG showed significant sensitivity to Headline. Interestingly, Headline, either solo or in combination with other fungicides and insecticides significantly (*P*<0.05) suppressed brown spot, and frogeye leaf spot across 11 seasons, even under low diseases pressure (Navi *et al*., 2015). Although, no significant advantage of Headline (solo or combination) in plots with SDS and WM, but significant (*P*<0.05) yield increase was observed over unsprayed controls indicating plant health benefits of spray (Navi *et al*., 2015).

Priaxor (fluxapyroxad + pyraclostrobin), as a foliar spray is effective against alternaria leaf spot, anthracnose, Asian soybean rust (not registered for use in California), brown spot, Cercospora blight, frogeye leaf spot, pod and stem blight, Rhizoctonia aerial blight, suppression only white mold and southern blight (*Sclerotium rolfsii*) as per the label. Out of this list, in *in vitro* test, Priaxor was significantly effective in reducing the growths of labeled (CT, RS, SG, and SS) and not labeled pathogens (FV, and MP) except PI compared with control (Plates-3 and 4). Priaxor field spray was also significantly effective against white mold and yields (Navi, 2014; 2014a) and significant effect on white mold but not on yields (Navi, 2013). 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 CT (not labelled) and RS (labelled) compared with control (Plates-3 and 4). At this point no speculation if Sercadis could be effective against CT and RS in field tests too.

According to Bayer CropScience, Stratego YLD (prothioconazole + trifloxystrobin) controls alternaria leaf spot, anthracnose, Asian soybean rust, brown spot, cercospora blight, frogeye leaf spot, pod and stem blight, powdery mildew, rhizoctonia aerial blight. Out of this list, *in vitro* results showed significant reduction in growth of both labeled (CT, RS, SG) and
not labeled pathogens (FV, MP, and SS) except PI compared with control (Plates-3 and 4). Foliar spray of Stratego YLD, did not show significant effect on white mold and yields (Navi, 2014), and on sudden death syndrome, white mold and yield (Navi, 2013). Also, field tests by Giesler (2012) indicated, no significant effect on brown spot severity and yield. In 2015 seed treatment tests with these products, some of the fungicides showed significant increase in stand count, suppression of FV and RS compared with control (Navi, 2015).