Original Research Article

In vivo bio-efficacy of fungicides and antagonists for the management of target leaf spot of soybean [*Corynespora cassiicola* (Berk. and Curt.) Wei]

Abstract

Foliar diseases cause major qualitative and quantitative yield loss in soybean, among which target leaf spot of soybean caused by *Corynespora cassiicola* is one of the constraints. Hence, management of this disease is much needed effort. Thus the present investigation was carried out for management of the disease under field condition using fungicides and bioagents that were effective in inhibiting the pathogen under *in vitro* studies. Among the different treatments evaluated, seed treatment with (pyraclostrobin 5 % + thiophanate methyl 45 %) @ 2 ml/kg seed followed by spray with (tebuconazole 50 % + trifloxystrobin 25 %) @ 0.05 % at 55 and 75 days after sowing (DAS) recorded the least disease severity of 2.23 percent with higher seed yield (17.20 q/ha) and test weight (17.41 g) as compared to untreated check which recorded disease severity of 27.50 percent. Apart from the target disease, other diseases like rust, anthracnose and Alternaria leaf spot were also take care in the same treatment. Use of fungicides for the management of disease in the absence of resistant genotypes is an old practice and it is one of the best options when there is outbreak.

Key words: Soybean, target leaf spot, *Corynespora cassiicola*, fungicides, antagonists, management

Introduction

"Soybean [*Glycine max* (L.) Merrill] is the most important leguminous oilseed crop grown worldwide, with seeds containing high amounts of both protein (40 %) and oil (20 %). The crop is considered as wonder crop, golden bean and miracle bean of 20th century. The origin of soybean domestication is thought to be in China but its great adaptability to different latitudes, climatic and soil conditions enabled soybean to become the fourth most widely grown crops across the globe after wheat, maize and rice. In India the crop is grown in an area of 12.20 million hectare with a production of 11.90 million tonnes and a productivity of 991 kg/ha" (Anon., 2022).

"Diseases are one of the major constraints for successful cultivation of soybean. The crop is known to be infected by more than 100 pathogens at various stages of crop growth

(Hartman *et al*., 1986) of which 35 are economically important including the most devastating disease like Asian rust" (Gupta, 2004). Now a days, some of the minor diseases like target leaf spot caused by *Corynespora cassiicola* (Berk. and Curt.) Wei are gaining importance in soybean growing areas of Karnataka. The disease target leaf spot of soybean was first reported during 1945 in USA (Olive *et al*., 1945) and now it has been found in most of the important soybean growing states. The disease has also been reported from different countries like Cambodia, Canada, China, Japan and Nicaragua (Sinclair, 1982). "In India, it was reported from Palampur during 1999-2000 and from Jabalpur during 2002-03" (Patel, 2005).

"The symptoms upon infection by *C. cassiicola* can be noticed on leaves, stems, pods and seeds. Leaf lesions are circular to irregular and appear reddish brown; they vary from small specks to big mature spots. Lesions are frequently surrounded by a dull green or yellowish green halo. Severely affected leaves drop prematurely" (Sinclair, 1982). "On stem and petiole the spots are dark brown and spindle shaped. On pods the spots are mostly circular with slightly depressed having light brown centre and dark brown margin. The pathogen can also survive in a fallow field for two years. Disease severity was significantly higher when humidity is more than 80 per cent" (Patel, 2005). "Dry weather conditions will suppress disease development. Optimum temperature for mycelial growth of the pathogen is 25-30^{0}C while conidial germination is maximum at 30^{0}C" (Kang *et al*., 1993).

The increase in the incidence of minor diseases may be related to change in weather parameters, change in pathogen virulence and introduction of varieties which are resistant to major diseases. From Karnataka, there are no reports or research work conducted on target leaf spot of soybean. Thus, keeping the view of wide occurrence of disease and its destructive nature, an attempt was made to develop an integrated strategy for management of the disease under field condition. "Use of foliar fungicides has not been a reliable control method for target leaf spot. But few fungicides are available for management of target leaf spot of soybean of which most used methyl benzimidazole carbamate (MBC) fungicide is carbendazim" (Ghini and Kimati, 2000).

Material and methods

Isolation and identification of target pathogen

"The infected plants showing typical symptoms of the disease were used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues of leaves were cut into small bits and were surface sterilized with 0.1 per cent sodium hypochlorite solution for 60 seconds and washed serially in sterilized distilled water and then transferred to sterilized Petri plates containing potato dextrose agar (PDA). Then the plates were incubated at room temperature (28 ^{0}C). The hyphal tips from margins of resulting colonies were cut with the help of sterilized 2 mm cork borer and transferred to Petri plates containing PDA" (Kurre, 2016).

Experimental site

The present field experiment was carried out during *kharif* 2018-19 at the experimental plot, AICRP on soybean, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, Karnataka, India. The experiment was conducted using variety JS 335 in a randomized block design (RBD) consisting of 8 treatments with three replications each.

Observations on bio-efficacy of fungicides and antagonists

Disease severity was assessed before spray and 10 days after spray, based on the disease severity score as given in the table below. The first spray was done after the first appearance of disease symptoms. The same concentrations were also followed during second spray applications with an interval of 20 days. The data on disease severity were recorded before spray application and 10 days after the spray application. Water sprayed plots served as control.

The observation of the disease on the foliage and disease severity was recorded by using 0-9 scale according to Mayee and Datar (1986).

Chart 1. Grade of the observation of the disease on the foliage and disease severity.

Grade	Description
0	No lesions
1	1 % leaf area covered with lesion
3	1.1 - 10 % leaf area covered with lesion
5	10.1 – 25 % of the leaf area covered no defoliation, little damage
7	25.1 – 50 % leaf area covered, some leaf drop, death of a few plant damage conspicuous

9	More than 50 % leaf area covered, lesion very common on all plants, defoliation common, death of plant common, damage more than 50 %.

Assessment of Percent Disease Index (PDI)

To record the severity of target leaf spot disease, 10 plants were selected randomly. Total 5 trifoliate leaves per plant were examined for visual symptoms of diseases. Percent disease index (PDI) was calculated by using the formula given by Wheeler (1969).

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of the individual rating}}{\text{Number of leaves observed} \times \text{Maximum grade}} \times 100$$

Observation on seed yield and test weight:

At the time of harvest, soybean seeds from all the treatments were collected and weighed separately and average weight was calculated. After threshing and winnowing, seed weight of each replication and test weight was recorded and yield per hectare was computed by using net plot yield data and it was then converted to quintal per hectare.

Statistical analysis

Statistical analysis was carried out for the data by the following procedure of Randomized Block Design. Calculations were made after applying the test of significance of the means. The per cent data of disease severity were transformed to Arc sine values.

Results

Symptomatology

The disease was noticed on all the above ground plant parts like leaves, stems and pods. On leaves spots appeared circular to irregular and dark brown in colour and size varied from small specks to big mature spots. These spots were surrounded by a dull green or yellowish green halo (Plate 1A, 1B and 1C). As disease progressed, the leaves turned yellow and dropped prematurely. On stem and petiole the spots are dark brown and spindle shaped (Plate 1D). On pods the spots were mostly circular with slightly depressed having light brown centre and dark brown margin (Plate 1E).

Isolation and morphological characteristics of the pathogen

Isolation of the pathogen was done from leaves showing typical symptoms of the disease target leaf spot. On the basis of morphological characters of mycelium and conidia, the pathogen was identified as *Corynespora cassiicola*. Under microscopic observation upon isolation, the mycelium was observed as septate, branched and pale brown in colour. Conidiophores raised singly from the mycelium which are septate and pale to dark brown in colour. Conidia formed either singly or in chains of 2-3 which are produced at the tip of the conidiophore. These conidia were slightly curved or straight having 8-10 pseudosepta and hilum at the base (Plate 2).

Management of soybean target leaf spot under field condition

A field experiment was carried out during *kharif* 2018-19 at the experimental plot, AICRP on Soybean, MARS, Dharwad for management of target leaf spot of soybean using fungicides and bioagents.

Eight different treatments were evaluated under field condition as discussed in Table 1. Among the treatments, T_3 [Seed treatment with (pyraclostrobin 5 % + thiophanate methyl 45 %) @ 2 ml/kg seed followed by spray with (tebuconazole 50 % + trifloxystrobin 25 %) @ 0.05 % at 55 and 75 DAS] recorded the least disease severity of 2.23 per cent with numerically higher seed yield of 17.20 q/ha and test weight of 17.41 g as compared to untreated check (PDI - 27.50 %, Yield - 9.04 q/ha, Test weight - 9.20 g) and was significantly superior to rest of the treatments. Along with target leaf spot, the treatment has also taken care of other foliar diseases like rust, alternaria leaf spot and anthracnose.

Seed treatment with (pyraclostrobin 5 % + thiophanate methyl 45 %) @ 2 ml/kg seed + spray with thiophanate methyl @ 0.1 % at 55 and 75 DAS (T_4) recorded PDI of 4.86 with yield of 16.03 q/ha and test weight of 16.42 g, followed by seed treatment with (carboxin 37.5 % + thiram 37.5 %) 2 g/kg seed + spray with thiophanate methyl @ 0.1 % at 55 and 75 DAS with 8.24 per cent disease severity and was statistically on par with T_7 *i.e.*, seed treatment with *Trichoderma harzianum* @ 5 g/kg seed + spray with (tebuconazole + trifloxystrobin) @ 0.05 per cent at 55 DAS and spray with *Pseudomonas fluorescens* @ 0.2 per cent at 75 DAS which recorded PDI of 12.12 per cent with a yield of 12.54 q/ha (Table 1 and Fig. 1).

Discussion

Corynespora cassiicola (Berk. and Curt.) Wei causing target leaf spot of soybean is having wide host range including crop and non-crop species. The MBC fungicides carbendazim and thiophanate-methyl have been used in the last decade to control target spot, anthracnose (*Colletotrichum truncatum* (Schw.) Andrus & Moore, 1935), Cercospora leaf blight (*Cercospora kikuchii* T. Matumoto & Tomoy, 1925) and Septoria brown spot (*Septoria glycines* Hemmi, 1915), but their efficacy has been decreasing year after year. There are several scientific proofs that deciphers the effectiveness of fungicides and biocontrol agents against soybean target leaf spot (Sloane et al. 1975; Conover, 1978; Mukherjee and Dasgupta, 1982; Rodriguez and Melendez, 1984; Mehrotra and Mehrotra, 2001; Manju et al. 2019). Xavier et al. (2013), studied the incidence of target leaf spot disease on soybean and observed increased incidence in recent years in Brazil even with intensive use of fungicides, and fungal resistance has been reported in recent studies. Similar work was carried out by Pernezny *et al.* (2002) for which they studied the management of target spot of tomato using different fungicides and reported that strobilurin group of fungicides like azoxystrobin found to be excellent in managing the disease. The results are also in confirmative with the findings of Choudhary *et al.* (2014) who reported that seed treatment with recommended combi product fungicides found best in managing the disease. Management of target leaf spot of soybean under field condition using six different fungicides as foliar spray among which Hexaconazole found most effective in managing the disease with disease severity of 33.67 per cent as compared to control (51.00 %). Mancozeb and pyraclostrobin found least effective with disease severity of 43.00 and 42.67 per cent respectively (Kurre, 2016). The combi product fungicide (pyraclostrobin 5 % + thiophanate methyl 45 %) is a combination of systemic and strobilurin group of fungicides, which is a new molecule using now a days against most of the seed borne diseases as a seed treating fungicide. Use of fungicides for the management of disease in the absence of resistant genotypes is an old practice and it is one of the best options when there is outbreak of disease but these fungicides need to be used judiciously based on their need, dose and type of disease to be managed.

Conclusion

The present investigation carried out for the evaluation of fungicides and bioagents concluded that the fungicide (pyraclostrobin 5 % + thiophanate methyl 45 %) as seed treatment followed by two sprays with (tebuconazole 50 % + trifloxystrobin 25 %) has given

good results in the effective management of soybean target leaf spot and also other foliar diseases like rust and anthracnose.

Declarations:

Consent

It was obtained from all individual participants included in the study. All the authors have seen and approved the manuscript, and all have taken a valid role through either study design, data generation or manuscript preparation.

Competing interests

No potential conflict of interest was reported by the authors.

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Table 1. Management of soybean target leaf spot (*Corynespora cassiicola*) through fungicides and antagonists under field condition

Treatments	Treatment Details	Per cent disease index	Per cent disease reduction over control	Test weight (g)	Yield (q/ha)
T_1	Seed treatment with (carboxin 37.5 % + thiram 37.5 %) 2 g/kg seed + spray with thiophanate methyl @ 0.1 % at 55 and 75 DAS	8.24 (16.68)*	70.03	12.40	14.48
T_2	Seed treatment with (carboxin 37.5 % + thiram 37.5 %) @ 2 g/kg seed + spray with carbendazim @ 0.1 % at 55 and 75 DAS	10.75 (19.11)	60.90	11.16	13.24
T_3	Seed treatment with (pyraclostrobin 5 % + thiophanate methyl 45 %) @ 2 ml/kg seed + spray with (tebuconazole 50 % + trifloxystrobin 25 %) @ 0.05 % at 55 and 75 DAS	2.23 (8.33)	91.89	17.41	17.21
T_4	Seed treatment with (pyraclostrobin 5 % + thiophanate methyl 45 %) @ 2 ml/kg seed + spray with thiophanate methyl @ 0.1 % at 55 and 75 DAS	4.86 (12.69)	82.32	16.42	16.03
T_5	Seed treatment with *Trichoderma harzianum* @ 5 g/kg seed + spray with *Pseudomonas fluorescens* @ 0.2 % at 55 and 75 DAS	19.94 (26.36)	27.49	10.16	10.60
T_6	Seed treatment with *Trichoderma harzianum* @ 5 g/kg seed + spray with carbendazim @ 1 g/l at 55 and 75 DAS	15.99 (23.55)	41.84	10.30	11.03
T_7	Seed treatment with *Trichoderma harzianum* @ 5 g/kg seed + spray with (tebuconazole 50 % + trifloxystrobin 25 %) @ 0.05 % at 55 DAS and spray with *Pseudomonas fluorescens* @ 0.2 % at 75 DAS	12.12 (20.35)	55.92	10.90	12.54
T_8	Untreated check	27.50 (31.61)	-	9.20	9.04
S. Em. ±		**1.28**	**-**	**0.10**	**0.75**
C. D. at 5 %		**3.88**	**-**	**0.32**	**2.29**
C.V.		**11.17**	**-**	**1.48**	**10.06**

*****Arc sine transformed values; DAS - Days After Sowing

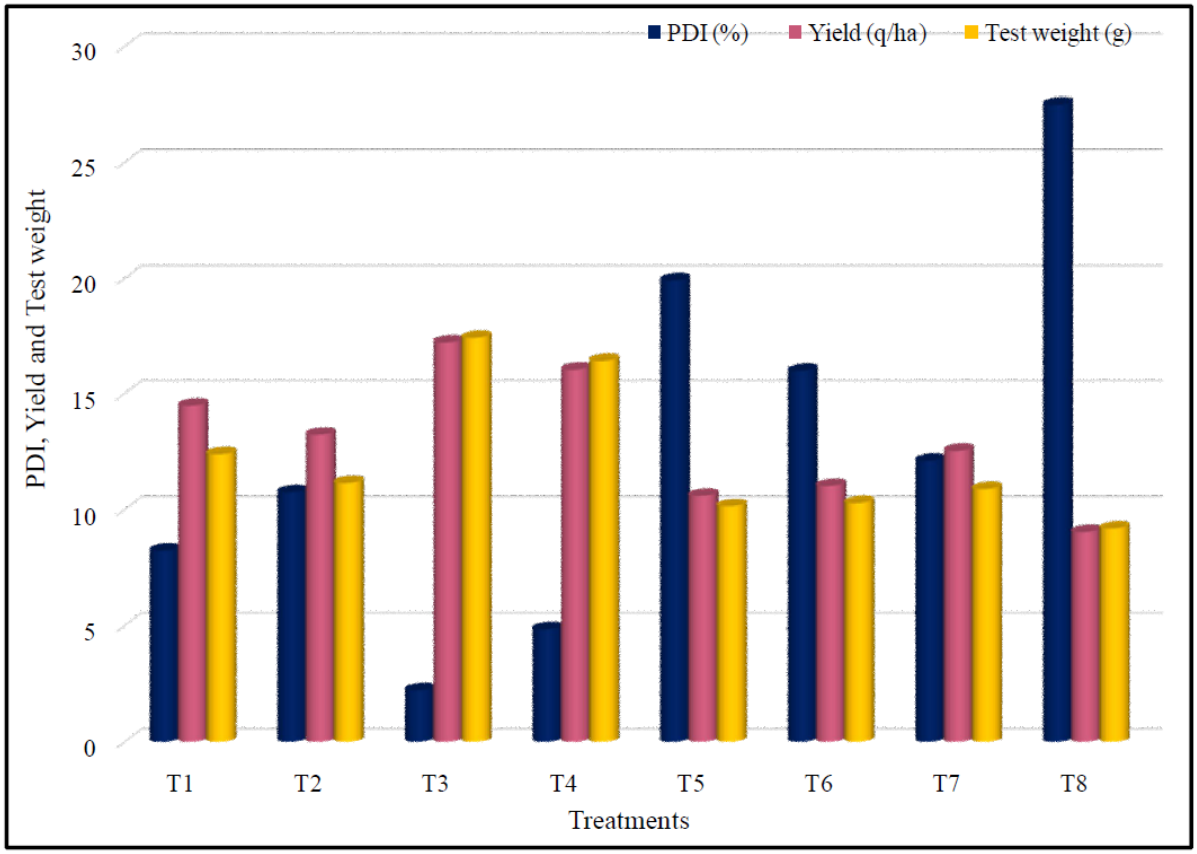

Fig. 1 Management of soybean target leaf spot (*Corynespora cassiicola*) through fungicides and antagonists under field condition

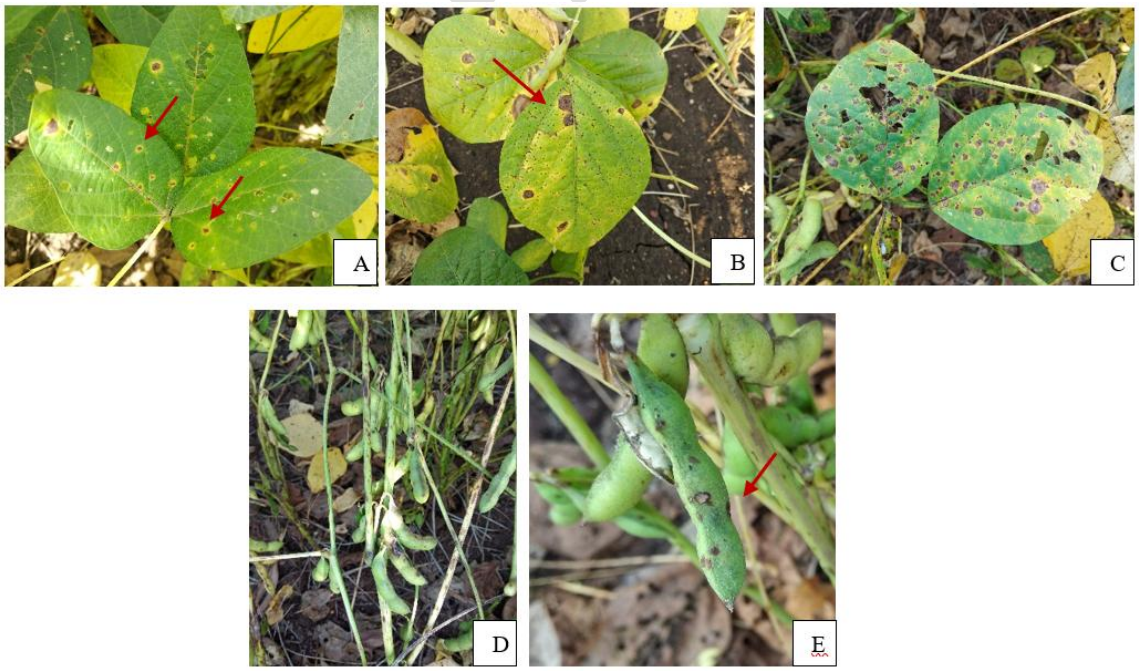

Plate 1. Symptomatology of target leaf spot on differet parts of soybean: A) Initial symptom, B) Dark brown spot with yellow halo on leaves, C) Severely infected leaves, D) Dark brown, spidle shaped spots on stem and petiole, E) Depressed spot with brown margin on pods.

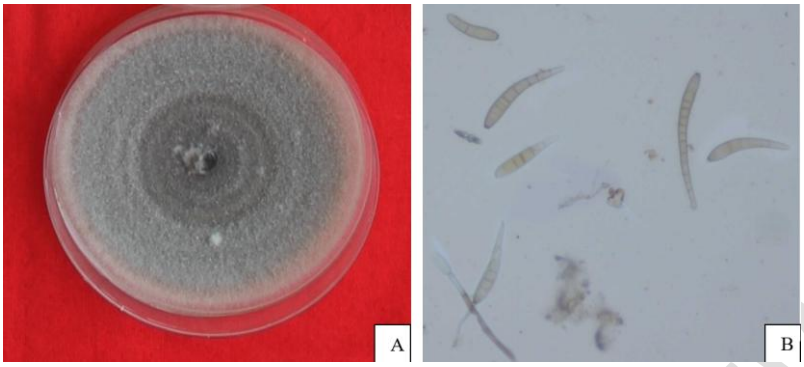

Plate 2. **Cultural and morphological characteristics of *Corynespora cassiicola***: A) Pure culture of *Corynespora cassiicola* on Potato Dextrose Agar, B) Conidia with conidiophore produced in culture plate.