Original Research Article

Integrated management of Charcoal rot and its influence on seed yield and quality in soybean

Abstract

Charcoal rot (*Macrophomina phaseolina*) causes high grain yield loss in top soybean-growing nations worldwide. The present study aimed to reveal the effect of stage-wise charcoal rot incidence and their root and stem severity index on the yield criteria of soybean varieties. Additionally, an attempt was made to derive integrated management practices and their influence on seed quality. All the twelve varieties were affected by charcoal rot, and its incidence and root and stem severity index were high in Shivalik (48.5% and 3.7, respectively). In yield estimation, the percentage of yield loss ranged from 8.7% (JS 20-98) to 53.9% (Shivalik). Per cent yield loss had a strong, significant positive relation with per cent incidence (0.912**) and Root and Stem severity index (0.813**) of charcoal rot. Seed treatment of Penflufen 13.28% + Trifloxystrobin 13.28 % FS @ 1ml/kg followed by spraying of tebuconazole 25% EC @ 0.1 % spraying at 45,60,75 days was significantly superior in reducing charcoal rot (19.7%) in comparison to untreated (37.3%). The highest seed germination (80.0 %) and least association *with M. phaseolina* (13.33%), *Aspergillus flavus* (3.33%), *Fusarium sp*., (13.34%) in comparison to control (50, 36.67, 23.33 and 36.67 %, respectively) were also resulted from the seed obtained from above treatment. In bioagent, seed treatment of *T. harzianum* @ 10 gm/kg followed by foliar spray of tebuconazole 25% EC @ 0.1% at 45,60,75 days was the second-best combination among all treatments in all respects. The effect of these fungicides and bio agents as seed treatment also improved the number of branches, pods, 100 seed weight and yield. Hence, these combinations could be applied to minimize charcoal rot and yield losses in soybean.

Key words: Associated Mycoflora, Charcoal rot, Incidence, Management and Yield loss

Introduction

Soybean (*Glycine max* (L.) Merrill), also designated as "Golden bean", is the predominant legume crop distributed across the world (Banerjee *et al*., 2022). It is one of the major oil crops worldwide. India ranks fifth in soybean oil production and consumption (Barela *et al.,* 2022). In India, the area is 12.7 Mha with an annual production of 10.45 Mt and a productivity of 0.82 tons per hectare as per the data for 2021-22 (USDA, 2022). Madhya Pradesh is a key soybean contributing state called "Soya state" or "Fort of Soybean". Its contribution to the national basket is more significant than 50% of area and production. It covers 54.01 lakh hectares and annually produces 6.69 million tonnes with a productivity of 1020 kg/ha (SOPA, 2021). Soybean is highly recognized and multipurpose utility food as it contains high protein (36.1-42.2%), balanced edible oil (16.8-20.2 %) and other valuable constituents (Kumar *et al.,* 2019; Uikey *et al.*,2022; Banerjee *et al.,* 2023; Jawarkar *et al.,* 2023). It is also one of the most suitable crops in crop rotation. It improves soil fertility by nitrogen fixation ability and economizes crop production for themselves and the next crop grown (Nassiuma and Wasike, 2002).

Soybean crop is affected by many economic diseases worldwide (Wrather *et al.*,2010). In India, the central part of the country that contributes around 90 per cent of soybean has been reported to be affected by several diseases(Amrate *et al.,* 2018; Amrate & Shrivastava, 2021; Amrate *et al.,* 2021a; Rajput *et al.,* 2021; Nataraj *et al.,* 2023). Among the fungal diseases, charcoal rot of soybean has been reported to cause epiphytotic in the U.S.A, China, Argentina, and Brazil (Wrather *et al.,*1997), and in India, it is also caused grain yield loss of Soybean (Wrether *et al.,*2010; Amrate *et al.,* 2019). The disease is common in central India, including in Madhya Pradesh, Maharashtra and Rajasthan (Amrate *et al.,*2023). Its pathogen, *Macrophomina phaseolina,* has a wide range, including about 500 different crop plant species and is reported to be soil, seed and stubble-borne (Luna *et al*., 2017; Amrate *et al*. 2023). Symptoms of charcoal rot are more common during the reproductive stage of the crop. The plant's initial dull greenish colour can recognize the disease, followed by rapid wilting. Even after wilting, the leaves may remain attached. Finally, the lower stem and taproot may appear charcoal-like greyish-black (Amrate *et al*., 2020a). This fungus survives through microsclerotia, which germinate to produce germ tubes and causes infection in emerging seedlings as well as in adult plants throughout the cropping season (Luna *et al*., 2017; Amrate *et al*., 2021b; Amrate *et al*., 2023). The fungus can survive for more than ten months under dry soil conditions.

Due to the very wide host range and necrotrophic nature of the pathogen, the disease is difficult to manage. Growing resistant or moderately resistant varieties is a very practical, economical, sound and environmentally friendly approach to managing charcoal rot

(Mengistu *et al*., 2007; Amrate *et al*., 2019; Amrate *et al*., 2023b). In addition to this, seed treatment with fungicides, biological control agents, plant extracts, soil solarization, chemical inducers, modification of planting dates, and crop rotation are also effective in minimizing the incidence of charcoal at some limit (Hewidy*et al*., 2003; Dubey *et al*., 2009; El-Baz, 2007; Luna *et al*., 2017). In the recent past, very high incidences of charcoal have been observed in varieties and several crucial genetic stocks, including an exotic collection of soybeans in Madhya Pradesh Agro-conditions (Amrate *et al*., 2020b; Amrate *et al*., 2023). The disease regularly occurs, but its severity depends on environmental conditions and the type of cultivar grown. Hence, a detailed and systemic study about stage-wise disease appearance and their impact on soybean yield is required. Besides this, management of the disease also needs to be worked out by using recent means. Therefore, the present study was carried out to determine the stage-wise appearance of disease and its influence on yield and to evaluate integrated management practices and their influence on seed quality parameters.

Material and Methods

Isolation and Identification of Pathogen

A plant showing typical symptoms of Charcoal rot was identified (Amrate *et al.,* 2020a). The associated pathogen was isolated on PDA using a standard isolation technique and identified based on a standard key (Barnett & Hunter, 1972).

Incidence of charcoal rot and Root and stem severity index

An experiment was conducted to assess the occurrence of charcoal rot in soybean and its effect on seed yield and quality. The experiment took place at the experimental area of AICRP on Soybean, Breeder Seed Production unit, JNKVV, Jabalpur during the Kharif season of 2022. This experiment was conducted in RBD while keeping two replications in a plot size of 3 x 0.9 m2 (three-row plot). Twelve varieties were sown in the last week of June. All the recommended practices were followed to raise the crop in the field. Observation of Charcoal rot incidence was taken at randomly selected plants at the flowering (R1-R2), pod development (R3-R4), seed development (R5-R6) and maturity stage (R7-R8). The following formula calculated the percent disease incidence.

$$\text{Percent disease incidence} = \frac{\text{No of plants death}}{\text{Total number of plants observed}} \times 100$$

Plants were also observed for microsclerotia and vascular discoloration at the same stages by splitting the stem longitudinally (1-5 rating) (Mengistu *et al.,* 2007). This root and stem severity (RSS) index of charcoal rot infection was taken from five randomly selected plants.

Percent Yield loss estimation

A similar set of twelve varieties was also evaluated for yield loss estimation due to charcoal rot. Five healthy plants, not affected by any other disease, were tagged and harvested separately. The expected yield of a healthy plot was calculated by multiplying the average yield of one healthy plant by the number of plants. In the end, the actual yield of the plot was taken. The plant showing other disease symptoms was avoided (Amrate *et al.,* 2019). The following formula calculated percent yield reduction.

$$\text{Percent yield reduction} = \frac{\text{Expected yield- Actual yield}}{\text{Expected yield}} \times 100$$

Integrated management of charcoal rot

Another field experiment evaluated the efficacy of various seed treatment treatments and foliar applications at different day intervals during *Kharif* 2022. Moderately susceptible variety JS - 20-29 was sown in randomized block design in the plot size of 3m x 2m (five rows plotted 40 cm line to line), keeping three replications. A total of 8 treatment combinations were applied, and sowing was done the first week of July. The percentage incidence of charcoal rot was recorded at 45, 60, 75, and 90 days after sowing, with a sample of 100 plants randomly selected from each treatment. Using the per cent mortality due to charcoal rot at 45,60,75 and 90 days, AUDPC (Area under disease progression curve) was calculated as given by (Shaner and Finney, 1977).

$$\text{AUDPC} = \sum_{i=1}^{n-i}[(yi + y_{i+1})/2][t_{i+1} - t_i]$$

Where,

yi = Percent incidence at i^{th} observation,

ti = time (days) at i^{th} observation, and

n= number of observations.

Other observations, such as the number of branches and pods, were counted from the five randomly selected plants from each treatment at harvesting time. Plot-wise, the yield was collected in gm and later converted into q/hectare for each treatment. The hundred seed weights were measured for each treatment.

Effect of treatment on seed quality

The seed obtained from previous field trials, treatment and replication wise, was also utilized to determine the influence of treatments on seed germination and association of the mycoflora seed quality. The collected replication seeds were sown in pots at 30^0C to 35^0C. After ten days, the seed was observed for germination.

$$\text{Percent seed germination} = \frac{\text{Total germinated seed}}{\text{Total number of seed sown}} \times 100$$

In another trial, a standard blotter test was carried out to detect mycoflora associated with seeds collected from previous year’s field trial (Neergaard,1979). The base of sterile plastic petri plates was covered with three layers of blotting paper (Whatman TM filter paper no. 1) soaked in sterile distilled water, ensuring enough. Soybean seeds were placed evenly spaced on petri dishes in aseptic conditions and incubated for seven days. The seeds were then scrutinized using a stereo binocular microscope on the seventh day. Fungal structures, such as conidia, conidiophores, and fruiting bodies, were examined to identify specific fungi. Identification was carried out under a compound microscope by placing a glass object containing fungal scrapings on the seeds (Ramdan *et al.*, 2022). The microflora associated with the tested seeds were counted and recorded. The percentage of infection by seed was calculated using the formula given below.

$$\text{Infection percentage} = \frac{\text{Infected seed}}{\text{Total seeds}} \times 100$$

All the data wherever required, transformed appropriately and analysed by using online software OP stat and SPSS 16.

Results and Discussion

Incidence and root and stem severity index of charcoal rot

The incidence of charcoal rot among all the varieties started in the seed development stage (R5-R6) and was high in the maturity stage (R7-R8). The distribution of charcoal rot among the varieties was statistically significant at both stages of seed development (R5-R6) and maturity stage (R7-R8) (Table 1). Before this, no incidence of charcoal rot was observed during flowering (R1-R2) and pod development stage (R3-R4). The percentage of incidence of charcoal rot ranged from 0.0% (HIMSO 1689, NRC 86, and JS 20-98) to 37.5% (Shivalik) at seed development. Variety Shivalik (48.5%) showed the highest per cent incidence, followed by JS 20-94(37.5%) at maturity (R7-R8) (Table 1). The lowest per cent incidence was found in variety JS 20-98 (3.0%) followed by NRC 86 (17.0%) at the maturity stage. In the root and stem severity index, in the early reproductive stage (Flowering and pod development), the root and stem severity of charcoal rot of all the varieties was 1.0, similar to healthy plants. The root and stem infection were visible in the seed development stage (R5-R6) and high in the maturity stage (R7-R8). Among all varieties, Shivalik (3.7) showed the highest root and stem severity of charcoal rot, followed by JS 20-94(3.1). The lowest root and stem severity of charcoal rot was found in JS 20-98 (1.2) followed by NRC 86 (1.6). Similar to the per cent incidence, the Root and stem severity index of charcoal rot varied significantly among varieties at both seed development and maturity stages (Figure 1).

In previous research, up to 100% incidence of charcoal rot was noticed in soybean varieties and germplasm (Amrate *et al.,* 2019; Amrate *et al.,* 2023). Singh and Bhowmick (1991) reported root rot incidence of up to 71.5 Percent in sesamum. Charcoal rot is a common, highly distributed disease in soybean, and its incidence was recorded by many researchers in different regions of the world (El- araby *et al.*, 2003; Mengistu *et al.,* 2011; Ansari, 2007; Bradly & Rio, 2003). The root and stem severity index is an important observation that can be utilized by identifying the plant affected by *M. phaseolina* without showing symptoms. Mengistu *et al*., (2007) described the criteria of root and stem severity index of charcoal rot and reported various levels of infection (1-5) in soybean. Other researchers also mentioned the root and severity index of charcoal rot (Mengistu *et al*., 2018; Coser *et al.,* 2017; Luna *et al.*, 2017).

Yield loss estimation

Among healthy plants of varieties, the highest yield was obtained from NRC 86 (7.2gm plant), followed by JS 20-98 (7.0 per plant) and JS 335 (6.4 per plant). The 100 seed weight ranged between 9.6 (NRC 7) to 11.6 (Punjab 1) in healthy plants of variety (Table 1). The yield was expected to range from 532.9 gm to 846.8 gm. Due to the attacks of charcoal rot in varying intensity, the actual yield was obtained from 247.5 gm to 773.0 gm. The actual 100 seed weight was obtained from 6.3gm (Shivalik) to 11.2 gm (JS 20-98). Charcoal rot in seed development and maturity stage were significantly affected yield and 100 seed weight. The highest yield reduction in variety Shivalik (53.9%) and 100 seed weight in variety JS 20-94 (41.6%) were recorded. The lowest reduction in yield and 100 seed weight in JS 20-98 of 8.7%and 2.3%, respectively, were recorded. Pearson correlation matrix was derived among the severity of charcoal rot (root and stem severity index, and per cent incidence), and yield parameters revealed that per cent yield loss had a solid significant positive relation with per cent incidence (0.912**) and Root and Stem severity index (0.813**) of charcoal rot (Table 2). Similarly, loss in 100 seeds was also significantly positively related to the per cent incidence (0.884**) and root and stem severity (0.888**) of charcoal rot. Previous to this, Amrate *et al*.,(2019) recorded 92.6% yield reduction in a soybean variety affected by charcoal rot disease.

Effect of treatments on percent incidence of charcoal rot and AUDPC

Results revealed that fungicidal treatments and bioagents significantly reduced charcoal rot incidence at 75 DAS and 90 DAS (Table 3). Among all treatments, seed treated with Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml/kg seed + spraying of Tebuconazole 25% EC @0.1% at 45,60,75 days (T3) recorded the lowest incidence and AUDPC (75 DAS, 4.3%)(90 DAS, 19.7%) (AUDPC, 212.5). It was found to be significantly superior to untreated control. Other treatment seed treatment with *Trichoderma harzianum*@10gm/kg seed + foliar spray with Tebuconazole25% EC @0.1% at 45,60,75DAS, (T6) (75 DAS, 8.0%) (90 DAS, 21.0%) (AUDPC 277.5) were also effective in reducing the incidence of charcoal rot significantly. The incidence of charcoal rot (13.7% at 75 DAS), (37.3%, 90 DAS) and AUDPC (485.0) was high in untreated. Several researchers also reported the efficacy of bioagents and fungicides against *M. phaseolina*. Elham *et al.* (2016) reported the effectiveness of three *Trichoderma harzianum* against charcoal rot in soybean (*Glycine max* L.). One of its isolates exhibited little plant disease indices in field studies, both in the soil incorporation (11.98%) and seed inoculation (5.55%) treatments.

Murthy et *al*. (2003) found increased germination over control of black gram against *M. phaseolina* and *Fusarium spp*.

Data showed that number of branches, pods were found significant better in (T6) seed treatment of *Trichoderma harzianum*@10gm/kg seed plus spraying of Tebuconazole 25% EC @0.1% at 45,60,75 DAS and hundred seed weight and yield (Q/ha) were also found significant better (T3) in Seed treatment of Penflufen 13.28% + Trifloxystrobin 13.2% FS @1ml/kg seed plus +spraying of Tebuconazole 25% @ 0.1% at 45,60, 75 DAS. In the case of untreated, all these parameters had the lowest value. Indra and Gayathri (2003) reported seeds treated with *Trichoderma* sp. (4g/kg seeds), the incidence of root rot caused by *M. phaseolina* was significantly reduced by 50%, and plant growth parameters such as root length, shoot length, grain yield, and nodulation were improved compared to control in black gram. Latha and Narasimhan (2006) recorded that Black gram seed treated with carbendazim (2g/kg) produced the highest pod production and the fewest roots compared to the control. Rajeshwari *et al*., (1999) reported the effectiveness of *T. harzianum* as a seed treatment and soil application in reducing the disease by 95.3%, increasing seed germination by 96%, increasing plant height by 35.5 cm, and increasing total biomass by 2.53 g/plant as opposed to control of dry root rot caused by *M. phaseolina* of green gram.

Effect of treatments on post-harvest germination of seed and associated seed mycoflora

Maximum seed germination was shown by seed treatment with Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml/kg plus spraying of tebuconazole 25% EC@ 0.1% at 45, 60, 75 days (T3) (80%) followed by (T2) seed treatment of Penflufen 13.28% + Trifloxystrobin 13.28 % FS @ 1ml/kg plus spraying of Tebuconazole 25% 0.1% at 45, 60 days and seed treatment with *T. harzianum* 10 gm/kg plus and foliar spray of Tebuconazole25% EC @0.1% at 45, 60, 75 days (T6) (76.67%) (Figure 2). Only seed treatment either of Penflufen 13.28% + Trifloxystrobin 13.28 % FS@1ml/kg (T7) (63.33%) or *T. harzianum* 10gm/kg seed (60.0%) had low seed germination. Fungicide spraying has resulted in improving germination and the quality of the seed. Significant differences in the occurrence of seed mycoflora were observed in variety JS20-29, and the results indicated that a total of 3 fungal species viz., *Macrophomina phaseolina, Aspergillus flavus,* and *Fusarium sp.* were detected (Figure 3). However, in most of the treatments, the highest seed mycoflora were found in untreated control (T9) *M.phaseolina* (36.67%), *A.flavus*,(23.33%) and *Fusarium* sp. (36.67%). Data showed that the lowest seed mycoflora were found in seed treatment with Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml/kg plus spraying of

Tebuconazole 25% EC0.1% at 45 ,60,75 days (T3) *M. phaseolina* (13.33%), *A. flavus*(3.33%), *Fusarium* sp. (13.34%) followed by and seed treatment with *T. harzianum*@10gm/kg seed plus foliar spray with Tebuconazole25% EC 0.1% at 45 ,60,75 days (T6) *Macrophomina phaseolina* (23.33%), *Aspergillus flavus*, (16.67%), *Fusarium* sp. (23.33%) respectively (Figure 2 and 3). Similar to this, Alemu *et al.* (2014) reported that a total of five fungi species comprising four genera, namely *Aspergillus flavus, Aspergillus niger, Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. from soybean seed. Ramesh *et al.*, (2013) examined and isolated *M. phaseolina, Fusarium spp., A. flavus, A. niger, Phoma*sp. and *Sclerotinia sclerotium* by using agar and standard blotter technique.

Conclusion

Our results have shown that using seed obtained from a previously treated field with fungicidal or bio agents, followed by a foliar application of fungicide, can significantly improve seed quality. This may be due to the combined effect of reducing pathogenic attacks and improving plant health, ultimately leading to better germination rates and a lower occurrence of other fungi in the seeds.

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Table 1: Incidence, and root and stem severity index of charcoal rot at Seed development (R5-R6) and maturity stage (R7-R8) of Soybean

	Seed development stage (R5-R6)		Maturity stage (R7-R8)		healthy plant		Expected plot yield (gm)	Actual		Yield loss (%)	100 Seed weight loss(%)
Variety	**Incidence (%)**	**R & S index**	**Incidence (%)**	**R & S index**	**Yield (gm)**	**100 seed weight (gm)**		**plot yield (gm)**	**100 SW (gm)**		
HIMSO 1689	0.0	1.0	18.0	1.9	6.2	10.6	676.1	533.0	9.2	20.8	13.1
NRC 86	0.0	1.0	17.0	1.6	7.2	11.0	753.8	604.8	9.5	19.7	14.0
JS 20-98	0.0	1.0	3.0	1.2	7.0	11.4	846.8	773.0	11.2	8.7	2.3
JS 20-94	17.5	1.3	37.5	3.1	5.9	11.0	642.3	360.3	6.4	43.9	41.6
JS 20-116	10.5	1.7	21.5	1.7	6.3	11.3	749.0	529.4	9.1	29.3	19.3
RVS 2001-14	18.0	1.8	27.0	2.9	5.5	11.0	656.3	517.5	8.3	21.4	24.3
AMS 100-39	5.0	1.3	18.0	2.6	6.3	10.6	723.6	519.1	7.7	28.2	27.2
JS 97-52	19.5	1.9	27.0	2.7	5.4	9.9	629.2	422.4	8.0	32.8	18.9
NRC 7	15.0	1.5	23.5	2.2	5.1	9.6	532.9	353.4	7.8	33.8	18.7
Shivalik	37.5	3.5	48.5	3.7	4.4	10.1	536.4	247.5	6.3	53.9	37.5
JS 335	13.0	1.2	21.5	2.1	6.4	10.6	678.3	525.0	8.6	22.6	18.4
Punjab 1	15.0	1.8	26.5	2.5	5.4	11.6	642.5	495.0	8.5	23.0	27.1
SE(m)	2.94	0.30	3.57	0.28	0.21	0.28	21.01	28.92	0.18	3.56	1.90
CD(p=0.05)	9.28	0.92	11.24	0.89	0.68	0.88	66.19	91.11	0.59	11.22	5.99

Table 2: Correlation between charcoal rot severity, incidence and yield parameters

	Yield and 100 seed weight obtained		Percent Yield and 100 seed weight loss	
	Yield	**100 seed weight**	**Yield**	**100 seed weight**
Charcoal rot incidence	-0.912**	-0.903**	0.912**	0.884**
Root and stem severity	-0.834**	-0.915**	0.813**	0.888*

Table 3. Effect of treatment on percent incidence of charcoal rot and growth parameters and yield of soybean

TN	Treatment details	Percent incidence		AUDPC	Number of branches	Number of pods	100 seed weight (gm)	Yield (Q/ha)
		75 DAS	90 DAS					
T1	ST - Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml + FA - Tebuconazole 25% EC @0.1% at 45 days	10.7	22.7	330.0	2.3	23.0	9.6	12.1
T2	ST-Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml + FA - Tebuconazole 25% EC @0.1% at 45,60 days	10.3	22.3	322.5	2.7	24.0	10.0	14.1
T3	ST-Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml + FA - Tebuconazole 25% EC @0.1% at 45,60 and 75 days	4.3	19.7	212.5	2.6	24.7	10.3	14.6
T4	ST – *T. harzianum* @10gm/kg seed +FA - Tebuconazole 25% EC @0.1% at 45 days	11.3	25.3	360.0	2.3	22.0	9.4	11.2
T5	ST – *T. harzianum* @10gm/kg seed +FA - Tebuconazole 25% EC @0.1% at 45, 60 days	9.7	25.0	332.5	2.3	21.7	9.6	11.8
T6	ST – *T. harzianum* @10gm/kg seed +FA - Tebuconazole 25% EC @0.1% at 45,60 and 75 days	8.0	21.0	277.5	2.7	26.0	9.6	12.7
T7	ST-Penflufen 13.28% + Trifloxystrobin 13.28 % FS @1ml/Kg	12.0	25.3	370.0	2.3	22.7	9.7	11.9
T8	ST – *T. harzianum*@10gm/Kg	12.7	26.7	390.0	2.0	20.3	9.4	11.2
T9	Untreated (control)	13.7	37.3	485.0	2.0	19.3	9.2	10.5
SE(m)	-	0.72	2.17	20.97	0.27	0.98	0.06	0.14
CD (p=0.05)	-	2.19	6.58	60.42	0.82	2.92	0.19	0.42

ST- Seed treatment **FA- Foliar application**

A B C D E

Figure 1: Depiction of Root and stem severity index of charcoal rot

A. No infection, blackening and microsclerotia absence **(Score =1)**
B. Slight infection, scattered microslerotia and vascular tissue discolouration is not prominent **(Score= 2)**
C. Moderate infection, vascular tissue discolouration and microsclerotia presence partially and clearly **(Score =3)**
D. Severe infection, numerous microsclerotia presence inside and outside of epidermis and vascular tissue **(Score=4)**
E. Very severe infection, complete blackening of internal and outer portion of root and stem with presence of numerous microsclerotia **(Score =5)**

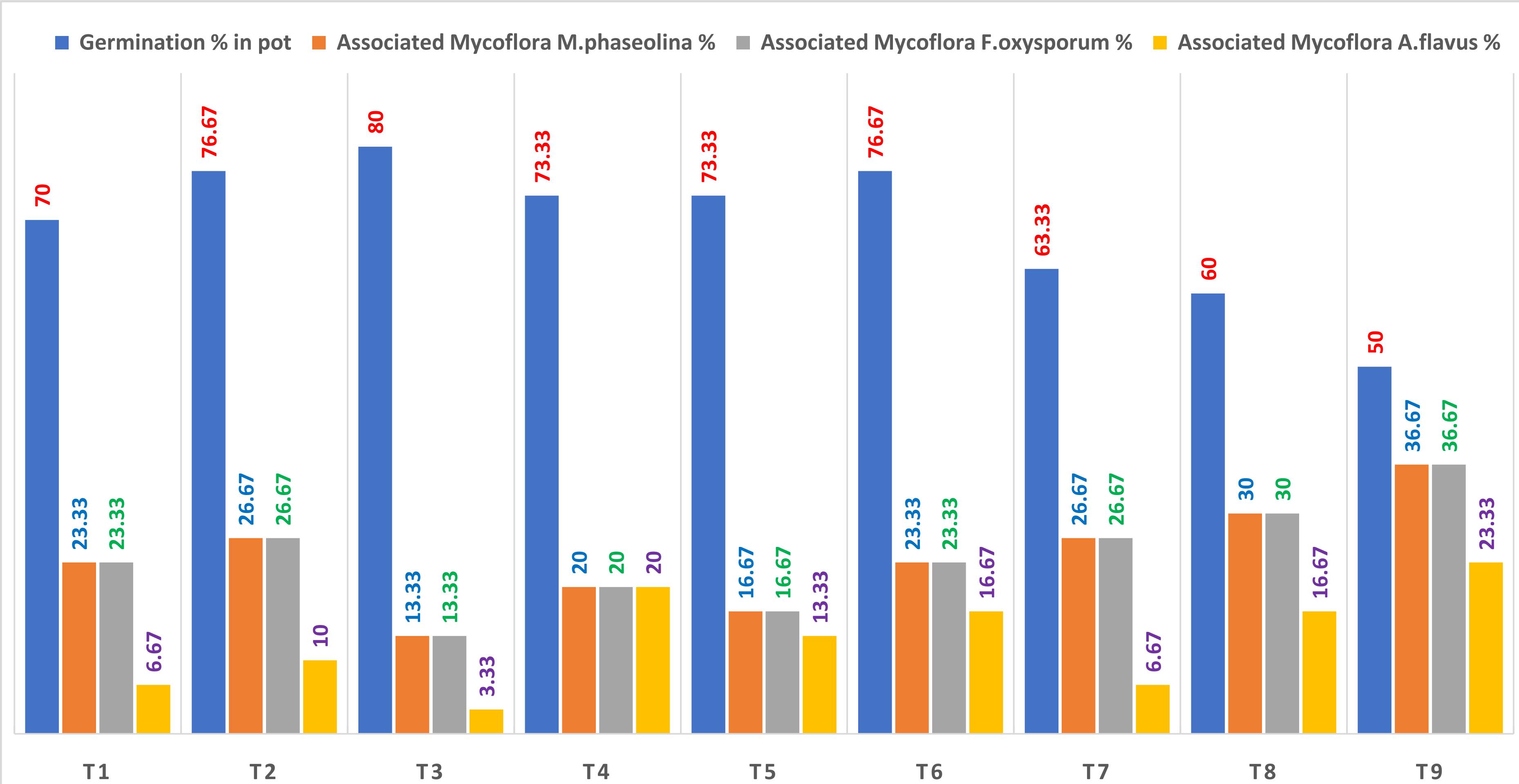

Figure 2: Effect of Field treatments on post-harvest germination of seed and associated mycoflora



T_1 T_2 T_3



T_4 T_5 T_6



T_7 T_8 T_9

Figure 3: Identification of seed associated mycoflora in seeds collected from field applied with different treatments