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Management of Seed Mycoflora of Yardlong Bean by Means of Seed Treatment with Fungicides *In Vitro* and *In Vivo*

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ABSTRACT: The present investigation was conducted during the year 2022-2023 at the Department of Plant Pathology, N. M. College of Agriculture, NAU, Navsari with an aim to manage seed mycoflora of yardlong bean using seed treatment of different fungicides. The study also recorded the data regarding seed germination (%), root length, shoot length and seedling vigour index for different treatments. For *in vitro* experiment paper towel method was used while *in vivo* experiment was conducted using pot method. Under *in vitro* condition trifloxystrobin 25 + tebuconazole 50WG found to be best which recorded highest seed germination 73.00 per cent, shoot length 12.69 cm, root length 12.14 cm and seedling vigour index 1811.32 followed by zineb 68 + hexaconazole 4WP (66.50%, 12.64 cm, 7.33 cm and 1327.94). Under *in vivo* condition metalaxyl 8 + mancozeb 64WP recorded highest seed germination 70.63 per cent, shoot length 22.21 cm, root length 5.04 cm and seedling vigour index 1923.33 followed by carbendazim 12 + mancozeb 63WP (70.00%, 19.21 cm, 3.76 cm and 1607.27).

Keywords: Seed mycoflora, yardlong bean, fungicides, shoot length, root length, seedling vigour index.

INTRODUCTION

Amid global challenges such as food insecurity, climate extremes, and rising living costs, beans offer a simple yet effective solution for ensuring nutritional and economic stability (Newnham, 2023). Beans, along with other legume vegetables, are widely consumed across the world for their high nutritional value. Botanically, they belong to the *Fabaceae* family (formerly *Leguminosae*), one of the largest families of flowering plants (Jin *et al.*, 2019). Based on modern classification, this family is divided into three sub-families, with *Papilionoideae*-particularly the tribe *Phaseoleae* and subtribe *Phaseolinae*- comprising nearly two-thirds of all known species (Gepts, 2001).

Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*), belonging to the *Leguminosae* family, is a tropical vegetable grown mainly for its long, tender green pods. Commonly known as Chinese long bean, snake bean, or bodi, its pods are typically about 1.5 feet long, despite the name "yardlong." It is also known as poor man's meat and is rich in protein content (23-32% of seed weight). It also contains vital vitamins like vitamin B, amino acids like lysine and tryptophan (Santhiya *et al.*, 2024). It thrives in warm climates up to 35°C and tolerates slightly acidic to neutral soils (pH 5.5–7.5), including heavy clay soils. It is widely cultivated in Southeast Asia, South China, and parts of India particularly Andhra Pradesh, Kerala, Karnataka, and Maharashtra- the crop covers around 18,560 to 20,160 hectares annually. In Gujarat, yardlong bean is a relatively new introduction, gaining popularity in kitchen gardens and isolated farming areas (Rachie, 1985; Devan *et al.*, 2021; Ano and Ubochi 2008; Anonymous, 2022; Anonymous, 2023).

Seed is a critical input in crop production, but it can also serve as a carrier of various pathogens, especially fungi, which are the most significant among seed-borne organisms. These pathogens may be present externally or internally and can cause serious diseases in the subsequent crop. Infected seeds lead to poor germination, reduced field establishment, lower market value, and significant yield losses. Fungal infections can result in seed rot, discoloration, abortion, and reduced seed size or viability. Such infections often occur during seed development, storage, or germination. Seed-borne pathogens can survive for long periods and spread across regions, making disease management challenging. Therefore, early detection

and rejection of infested seeds through seed health testing is essential. The risk and impact of seed-borne diseases vary depending on the crop, pathogen, and environmental conditions (Mehrotra and Aggarwal 2003; Akranuchat *et al.*, 2007; Amza, 2018; Kumar *et al.*, 2020).

Seed mycoflora of asparagus vigna (*Vigna unguiculata* (L.) Walp.) *viz.*, *Fusarium* spp., *Mucor mucedo* Sowerby, *Penicillium* spp., *Rhizopus* spp., *Aspergillus niger* Tiegh., *Alternaria* spp., *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* (Lib.) de Bary reported to contaminate the seeds (Fotev and Kazakova 2020). Seed mycoflora which are described earlier can deteriorate the quality of yardlong bean seeds which, directly effects the yardlong bean cultivation. So, it is better to take some control measures to prevent deterioration. Seed treatment with fungicides serves as a reliable strategy for managing seed-borne diseases, while also promoting seed health, better crop establishment, and increased yields (Tanweer, 1982). In any seed production system, proper post-harvest storage until the next sowing season is essential to preserve seed viability and vigour. Among the various disease management practices, seed treatment stands out as one of the most effective for controlling seed-borne fungal infections. The use of fungicides has proven successful in mitigating fungal threats to crops. Chemical seed treatments are considered both environmentally friendly and cost-effective, as they require only minimal quantities of active ingredients typically around 1 to 1.5 g per kg of seed compared to the higher doses used in foliar sprays (Ayesha *et al.*, 2021).

MATERIALS AND METHODS

A. Detection, isolation, purification, identification, and maintenance of the fungus cultures

Isolation of external and internal seed borne mycoflora was conducted by following standard International Seed Testing Association (ISTA) methods: (i) Agar Palte method (ii) Standard Blotter method (iii) Deep Freezing method. After the isolation of the fungi culture they were subjected to purification by hyphal tip method and after that identified by compared with the characteristics described in various published literature and identification was done by referring authentic relevant literature (Neergaard, 1977; Kanapathipillai, 1982; Ahmed and Reddy 1993; Thakur *et al.*, 2010; Fotev and Kazakova 2020). Cultures were maintained on PDA slants by sub culturing and stored at 5°C for further study.

B. Management of Seed Infecting Fungi by Means of Seed Treatment with Fungicides In Vitro

Different fungicides were evaluated to check their effect on germination and vigour index of seeds inoculated with isolated fungi. For this, healthy yardlong bean seeds were inoculated with the mixture of all isolated fungus by soaking the seeds into mixed spore suspension of fungi containing 1×10^6 cfu/ml and then, treated with all respective fungicides as mentioned in Table 1. These treated seeds were evaluated by Paper towel method (Khare, 1996) after incubated at 25±2°C for 10 days. After end of incubation period, observations were recorded as number of germinated seeds, shoot length and root length to calculating vigour index and germination percentage.

Table 1: Fungicides tested against seed infecting fungi of yardlong bean.

Sr. No.	Treatment Details	Conc. (%)	Dose
1.	Carboxin 37.5 + Thiram 37.5DS	0.22	3.0g/kg
2.	Azoxystrobin 18.2 + Difenoconazole 11.4SC	0.09	3.0ml/kg
3.	Captan 70 + Hexaconazole 5WP	0.22	3.0g/kg
4.	Carbendazim 12 + Mancozeb 63WP	0.22	3.0g/kg
5.	Metalaxyl 8 + Mancozeb 64WP	0.22	3.0g/kg
6.	Trifloxystrobin 25 + Tebuconazole 50WG	0.22	3.0g/kg
7.	Zineb 68 + Hexaconazole 50WP	0.22	3.0g/kg
8.	Absolute control (without treatment)	—	—

C. Management of Seed Infecting Fungi by Means of Seed Treatment with Fungicides In Vivo

The experiment was conducted at Navsari Agricultural University, Navsari during 2022-2023 on yardlong bean in pot method. It included eight treatments in a completely randomized design with four replications. Different fungicides were evaluated to check their effect on germination and vigour index of seeds inoculated with isolated fungi. For this, healthy yardlong bean seeds were inoculated with the mixture of all isolated fungus by soaking the seeds into mixed spore suspension of fungi containing 1×10^6 cfu/ml and then, treated with all respective fungicides as mentioned in Table 1. The treated seeds were sown in 18.5cm diameter plastic pots containing sterilized soil at the rate of ten seeds per pot and four replications were kept for each treatment. The pots were kept in polyhouse and watered regularly. After 21 days period, observations were recorded as number of germinated seeds, shoot length and root length to calculating vigour index and germination percentage.

D. Statistical Analysis

The data, collected under study were subjected to the statistical analysis for proper interpretation. The standard method of analysis of variance technique appropriate to the Completely Randomized Design (CRD) as described by (Panse and Sukhatme 1967) was used. The data were analyzed with the technical help received from Department of Agril. Statistics, N. M. College of Agriculture, NAU, Navsari. The treatment

differences were tested by employing 'F' test at five per cent level of significance on the basis of null hypothesis. The appropriate standard error of mean (SEm) was calculated and the critical difference (CD) at five per cent level of probability were worked out to compare the two treatment means, where the treatment effects were found significant under F test. The coefficient of variation percentage (CV %) was also worked out for all the cases to understand variability present in experimental unit.

RESULT AND DISCUSSION

A. Detection, isolation, purification, identification and maintenance of yardlong bean seed infecting fungi

Seed-borne mycoflora from composite sample of yardlong bean seeds were isolated by agar plate method, standard blotter method and deep-freezing method by surface and without surface sterilized seeds which revealed the association of ten different predominant fungi. The different isolates *viz.*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp., *Rhizopus* sp., *Fusarium* sp., *Colletotrichum* sp., *Curvularia* sp., *Alternaria* sp., *Botryodiplodia* sp. and *Sclerotium* sp. obtained from yardlong bean seeds were purified by hyphal tip and single spore isolation technique. After purification, each of the isolate was identified on the basis of their cultural and morphological characteristics described in various authentic relevant published literature.

B. Management of seed infecting fungi by means of seed treatment in vitro

Different seven fungicides were tested to check their efficacy on seed germination and seedling health of yardlong bean seeds inoculated with mixture of all isolated fungi under laboratory condition. Table 2 and Fig. 1 revealed significant effect of all fungicides on seed germination, shoot length, root length and seedling vigour index.

Table 2: Management of seed infecting fungi of yardlong bean by means of seed treatment by fungicides *in vitro*.

Treatment and Conc. (%)	Seed germination (%)*	Increase in seed germination over healthy seed (%)	Shoot length (cm)*	Increase in shoot length over healthy seed (%)	Root length (cm)*	Increase in root length over healthy seed (%)	Seedling vigour index (SVI)
T_1- Carboxin 37.5 + Thiram 37.5DS (0.22%)	59.75^{c}	58.27	12.84^{c}	4.30	7.80^{d}	12.88	1232.32^{c}
T_2-Azoxystrobin 18.2 + Difenoconazole 11.4SC (0.09%)	49.50^{e}	31.13	12.53^{cd}	1.79	7.10^{ef}	2.75	971.74^{e}
T_3-Captan 70 + Hexaconazole 5WP (0.22%)	41.50^{f}	9.93	12.62^{cd}	2.52	7.06^{ef}	2.17	816.73^{f}
T_4-Carbendazim 12 + Mancozeb 63WP (0.22%)	44.00^{f}	16.56	14.56^{a}	18.27	10.41^{b}	50.65	1100.78^{d}
T_5-Metalaxyl 8+ Mancozeb 64WP (0.22%)	53.50^{d}	41.72	13.34^{b}	8.37	9.46^{c}	36.90	1218.9^{c}
T_6-Trifloxystrobin25 + Tebuconazole 50WG (0.22%)	73.00^{a}	93.38	12.69^{cd}	3.08	12.14^{a}	75.68	1811.32^{a}
T_7-Zineb 68 + Hexaconazole 4WP (0.22%)	66.50^{b}	76.16	12.64^{cd}	2.68	7.33^{e}	6.07	1327.94^{b}
T_8-Control	37.75^{g}	—	12.31^{d}	—	6.91^{f}	—	725.88^{g}
SEm ±	1.12		0.13		0.10		23.54
CD at 5%	3.26		0.37		0.30		68.72
CV%	4.20		1.99		2.43		4.09
* Average of four repetitions							

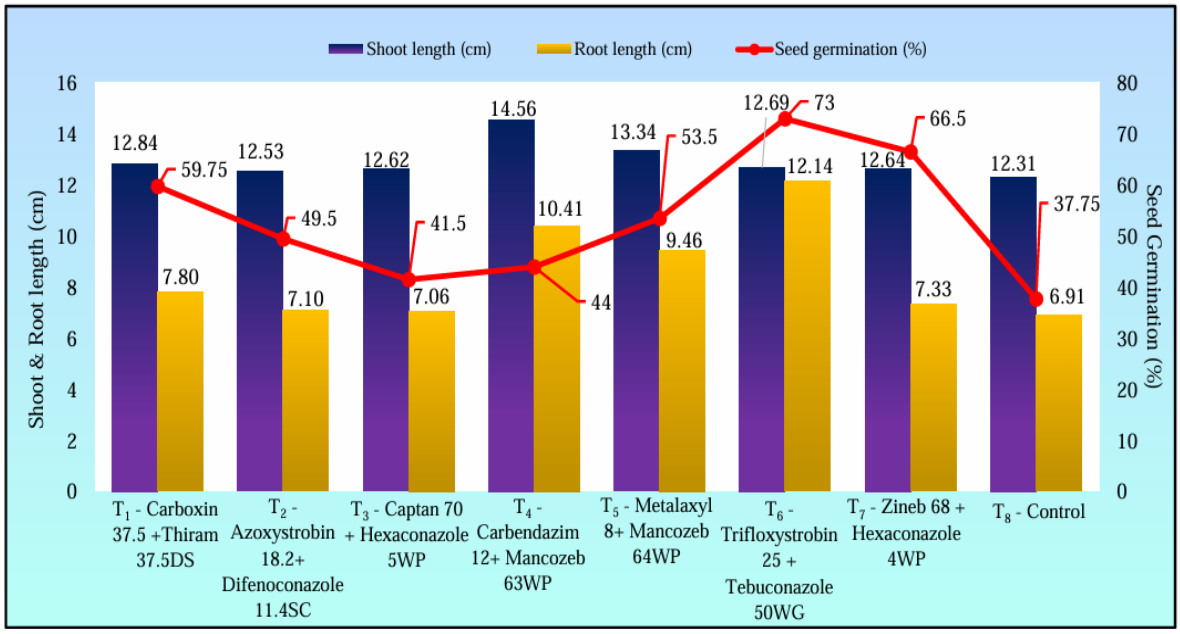

Fig. 1. Management of seed infecting fungi of yardlong bean by means of seed treatment with fungicides *in vitro*.

Overall, fungicides recorded 9.93 to 93.38, 1.79 to 18.27, 2.17 to 75.68 per cent increase in seed germination, shoot length and root length, respectively over control. Seed treated with trifloxystrobin 25 + tebuconazole 50WG recorded significantly higher seed germination (73.00%) over all treatments followed by zineb 68 + hexaconazole 4WP (66.50%). Whereas carboxin 37.5 + thiram 37.5DS recorded (59.75%) seed germination followed by metalaxyl 8+ mancozeb 64WP (53.50). Whereas azoxystrobin 18.2 + difenoconazole 11.4SC recorded 49.50 per cent seed germination. Seed treated with carbendazim 12 + mancozeb 63WP and captan 70 + hexaconazole 5WP recorded 44.00 and 41.50 per cent seed germination which was statistically at par with each other. Least seed germination 37.75 per cent was recorded in control.

Result in term of shoot length showed that seed treated with carbendazim 12 + mancozeb 63WP recorded significantly higher shoot length (14.56 cm) over the other treatments followed by metalaxyl 8+ mancozeb 64WP (13.34 cm). While carboxin 37.5 + thiram 37.5DS, trifloxystrobin 25+ tebuconazole 50WG, zineb 68 + hexaconazole 4WP, captan 70 + hexaconazole 5WP and azoxystrobin 18.2 + difenoconazole 11.4SC recorded 12.84, 12.69, 12.64, 12.62 and 12.53cm shoot length which had no significant difference among them. Least shoot length (12.31 cm) was recorded in control.

While in case of root length trifloxystrobin 25+ tebuconazole 50WG recorded significantly higher root length (12.14 cm) among all the treatment followed by carbendazim 12 + mancozeb 63WP (10.41 cm). Whereas, metalaxyl 8+ mancozeb 64WP recorded 9.46cm root length followed by seed treated with carboxin 37.5 + thiram 37.5DS (7.80cm). Seed treated with zineb 68 + hexaconazole 4WP, azoxystrobin 18.2 + difenoconazole 11.4SC and captan 70 + hexaconazole 5WP recorded 7.33, 7.10 and 7.06cm root length, respectively over control (6.91 cm). Maximum seedling vigour index was recorded in the seed treated with trifloxystrobin 25 + tebuconazole 50WG (1811.32) followed by zineb 68 + hexaconazole 4WP (1327.94). Whereas carboxin 37.5 + thiram 37.5DS (1232.32) and metalaxyl 8 + mancozeb 64WP (1218.9) were at par with each other. Whereas carbendazim 12 + mancozeb 63WP, azoxystrobin 18.2 + difenoconazole 11.4SC and captan 70 + hexaconazole 5WP recorded 1100.78, 971.74 and 816.73 seedling vigour index, respectively over control (725.88).

The above results are less or more in similarity with Chaudhari *et al.* (2017) tested the efficacy of seven fungicides *viz.*, mancozeb 75WP, carbendazim 50WP, metalaxyl 8 + mancozeb 64WP, pyraclostrobin 5 + metiram 55WG, carbendazim 12 + mancozeb 63WP, carboxin 75WP and chlorothalonil 75WP on germination and vigour index of seeds inoculated with isolated fungi from pigeon pea seeds *in vitro*. The treatment with metalaxyl 8 + mancozeb 64WP showed 93.33 per cent average seed germination and seedling vigour index 2323.73 which was at par with carbendazim 12+ mancozeb 63WP which showed 92.00 per cent average seed germination and 2201.07 seedling vigour index. Whereas, Sunil Kumar *et al.* (2021) evaluated efficacy of seed dressing fungicides, bioagent and priming agents against pod blight complex of soybean by rolled towel method *in vitro* using nine treatments. Result showed that the least per cent seed infection of 7.33 per cent was noticed in seed treated with carboxin 37.5 + thiram 37.5WP @ 2 g/ kg of seed showed 91.67 per cent germination and 785.11 seed vigour index which was found statistically at par with seed treated with seed treated with mancozeb 50 + carbendazim 25WP @ 2g/kg of seed which showed 10 per cent infection, 89.33 per cent seed germination and 708.04 seed vigour index. Pooja *et al.* (2024) treated green gram seeds with fungicides by using paper towel method to evaluate influence of treatments on seed quality attributes of green gram and found that seed treatment with mancozeb 50% WP+ carbendazim 25%

WS (Sprint) @ 2g/kg recorded significantly highest germination (84.67%), lower seed infection (12.00%) and showed 13.48 per cent increase in germination and 44.67 per cent decrease in infection over control.

C. Management of seed infecting fungi by means of seed treatment in vivo

Different seven fungicides *viz.*, carboxin 37.5 + thiram 37.5DS, azoxystrobin 18.2 + difenoconazole 11.4SC, captan 70 + hexaconazole 5WP, carbendazim 12 + mancozeb 63WP, metalaxyl 8+ mancozeb 64WP, trifloxystrobin 25+ tebuconazole 50WG and zineb 68 + hexaconazole 4WP were tested to check their efficacy on seed germination and seedling health of yardlong bean seeds inoculated with mixture of all isolated fungi under *in vivo* condition. Table 3 and Fig. 2 revealed significant effect of all fungicides on seed germination, shoot length, root length and seedling vigour index.

Table 3: Management of seed infecting fungi of yardlong bean by means of seed treatment by fungicides *in vivo*.

Treatment and Conc. (%)	Seed germination (%)*	Increase in seed germination over healthy seed (%)	Shoot length (cm)*	Increase in shoot length over healthy seed (%)	Root length (cm)*	Increase in root length over healthy seed (%)	Seedling vigour index (SVI)
T_1- Carboxin 37.5 + Thiram 37.5DS (0.22%)	51.25^{b}	32.26	15.62^{c}	13.6	3.67^{c}	40.07	986.94^{c}
T_2-Azoxystrobin 18.2 + Difenoconazole 11.4SC (0.09%)	44.38^{d}	14.53	14.23^{d}	3.49	5.13^{a}	95.80	858.10^{e}
T_3-Captan 70 + Hexaconazole 5WP (0.22%)	48.75^{bc}	25.81	14.27^{d}	3.78	4.77^{b}	82.06	927.69^{cd}
T_4-Carbendazim 12 + Mancozeb 63WP (0.22%)	70.00^{a}	80.64	19.21^{b}	39.71	3.76^{c}	43.51	1607.27^{b}
T_5-Metalaxyl 8+ Mancozeb 64WP (0.22%)	70.63^{a}	82.27	22.21^{a}	61.53	5.04^{a}	92.36	1923.33^{a}
T_6-Trifloxystrobin25 + Tebuconazole 50WG (0.22%)	46.88^{cd}	20.98	13.93^{d}	1.31	3.75^{c}	43.12	828.72^{e}
T_7-Zineb 68 + Hexaconazole 4WP (0.22%)	45.00^{cd}	16.13	14.34^{d}	4.29	5.17^{a}	97.32	878.42^{de}
T_8-Control	38.75^{e}	—	13.75^{d}	—	2.62^{d}	—	633.79^{f}
SEm ±	0.09		0.29		0.09		21.62
CD at 5%	0.26		0.85		0.26		63.10
CV%	4.27		3.64		4.27		4.00
* Average of four repetitions							

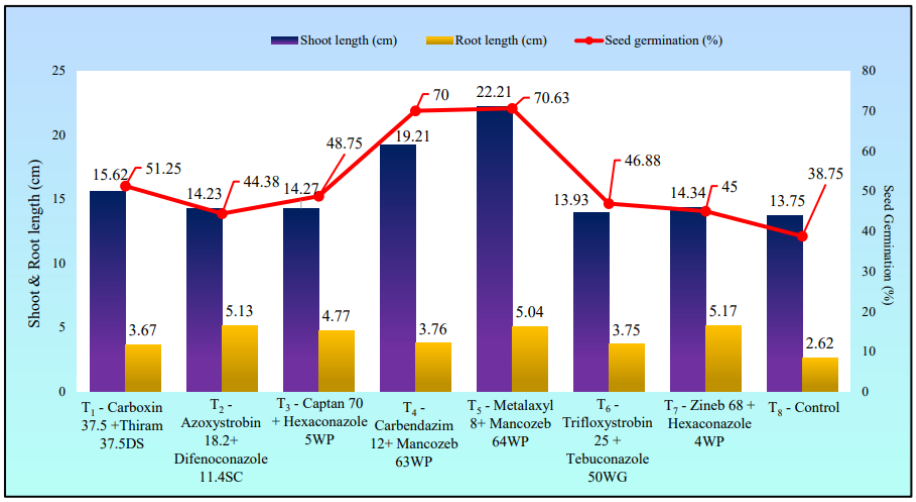

Fig. 2. Management of seed infecting fungi of yardlong bean by means of seed treatment with fungicides *in vivo.*

Overall, fungicides recorded 14.53 to 82.27, 1.31 to 61.53, 40.07 to 97.32 per cent increase in seed germination, shoot length and root length, respectively over control. Seed treated with metalaxyl 8 + mancozeb 64WP recorded significantly higher seed germination (70.63%) which was at par with carbendazim 12 + mancozeb 63WP (70.00%). Next best treatment in the merit of result was carboxin 37.5 + thiram 37.5DS (51.25%). Seed treated with captan 70 + hexaconazole 5WP, trifloxystrobin 25 + tebuconazole 50WG, zineb 68 + hexaconazole 4WP, azoxystrobin 18.2 + difenoconazole 11.4SC recorded 48.75, 46.88, 45.00 and 44.38 per cent seed germination which showed no significant difference among them. Lowest seed germination (38.75%) was recorded in control.

Result in term of shoot length seed treated with metalaxyl 8 + mancozeb 64WP recorded significantly higher shoot length (22.21 cm) followed by carbendazim 12 + mancozeb 63WP (19.21cm). While carboxin 37.5 + thiram 37.5DS recorded (15.61cm). Seed treated with zineb 68 + hexaconazole 4WP, captan 70 + hexaconazole 5WP, azoxystrobin 18.2 + difenoconazole 11.4SC and trifloxystrobin 25 + tebuconazole 50WG recorded 14.34, 14.27, 14.23 and 13.93cm shoot length which showed no significant different among them along with control (13.75 cm).

While in case of root length zineb 68 + hexaconazole 4WP recorded highest root length (5.17 cm) which was statistically at par with azoxystrobin 18.2 + difenoconazole 11.4SC (5.13 cm) and metalaxyl 8+ mancozeb 64WP (5.04cm) followed by captan 70 + hexaconazole 5WP (4.77cm). Seed treated with carbendazim 12 + mancozeb 63WP, trifloxystrobin 25+ tebuconazole 50WG and carboxin 37.5 + thiram 37.5DS recorded 3.76, 3.75 and 3.67cm root length had no significant difference among them. Lowest root length was recorded in control (2.62 cm). Highest seedling vigour index was recorded in the seed treated with metalaxyl 8 + mancozeb 64WP (1923.33) followed by carbendazim 12 + mancozeb 63WP (1607.27). Whereas carboxin 37.5 + thiram 37.5DS, captan 70 + hexaconazole 5WP, zineb 68 + hexaconazole 4WP, azoxystrobin 18.2 + difenoconazole 11.4SC and trifloxystrobin 25+ tebuconazole 50WG recorded 986.94, 927.69, 878.42, 858.10 and 828.72 seedling vigour index, respectively over control (633.79).

Results are less or more in similarity with Kharte *et al.* (2021) conducted a field experiment to evaluate the effect of seed treatment and foliar application of fungicides for the management of diseases of pea. Among the eight fungicides used, carbendazim + mancozeb 75WP @ 0.30 per cent recorded highest mean seed germination (78.2%), plant height (41.0 cm), shoot/plant (13.6 cm), pod length (6.58 cm), highest seeds/pod (6.98) and very lowest was observed in untreated control. Khillare *et al.* (2021) tested the effect of fungicides and bioagents against *Fusarium oxysporum* f. sp. *udum* on seed germination and seedling vigour in cultivar ICP-2376 in pot culture. The results revealed that, maximum seed germination per cent 96.90 per cent was observed in carboxin 37.5 + thiram 37.5WP @ 0.25 per cent followed by carbendazim 12 + mancozeb 63WP @ 0.25 per cent which recorded 93.80 per cent germination, least germination per cent was observed in control (untreated) which recorded 53.16 per cent germination. The maximum seedling vigour index was observed in carboxin 37.5 + thiram 37.5WP @ 0.25 per cent which recorded 4180.2 over control.

CONCLUSIONS

A total of ten fungal species were isolated using three methods: the agar plate method, the standard blotter method, and the deep-freezing method. Yardlong bean seeds were treated with different fungicides under *in vitro* and *in vivo* cinditions to check their effect on seedling vigour index. Among the seven fungicidal seed treatments evaluated under *in vitro* condition, trifloxystrobin 25 + tebuconazole 50WG emerged as the most effective treatment. It recorded the highest values for seed germination (73.00%), shoot length (12.69cm), root length (12.14cm) and seedling vigour index (1811.32), followed by zineb 68% + hexaconazole 4WP, which showed 66.50 per cent germination, 12.64cm shoot length, 7.33cm root length, and a seedling vigour index of 1327.94.Under *in vivo* condition, metalaxyl 8 + mancozeb 64WP recorded the highest seed germination (70.63%), with a shoot length of 22.21cm, root length of 5.04cm and a seedling vigour index of 1923.33. This was followed by carbendazim 12 + mancozeb 63WP, which showed 70.00 per cent germination, 19.21 cm shoot length, 3.76 cm root length, and a seedling vigour index of 1607.27.

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