The Bioscan 15(3):339-344, 2020
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EFFECT OF FUNGICIDES, PLANT EXTRACTS AND BIOAGENTS ON SPORE GERMINATION OF COLLETOTRICHUM LINDEMUTHIANUM CAUSING FIELD BEAN ANTHRACNOSE

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KEYWORDS
Anthracnose
Bioagents
Field bean
Fungicides

Received on :
18.05.2020

Accepted on :
12.08.2020

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ABSTRACT
Five fungicides at three different concentrations (0.05, 0.1 and 0.15%), two leaf extracts at three different concentrations (5, 7.5 and 10%) and cultural filtrates of three bioagents were evaluated in vitro by slide germination techniques to know their efficacy against conidia of Colletotrichum lindemuthianum causing anthracnose of field bean. All the treatments were found effective and recorded significant reduction in spore germination of the test pathogen over untreated control. However, in vitro studies revealed that, significant inhibition of spore germination was observed at higher concentration fungicides and leaf extracts. Amongst fungicides, propiconazole recorded lowest spore germination (12.93%) corresponding highest inhibition (85.44%) at 0.15%, while highest spore germination (42.30%) corresponding minimum inhibition (52.57%) was noticed at 0.05% of azoxystrobin. With respect to two leaf extracts with three concentrations each, Lantana camara at 10% concentration, inhibit the spore germination by 60.38%, which was statistically on par with 7.5% concentration of L. camara (57.39%). The highest spore germination (46.60%) was recorded at 5.0 per cent concentration of A. indica. Out of three bioagents, Trichoderma viride showed highest inhibition of spore germination (53.51%) followed by Bacillus subtilis (51.16%), while least inhibition was by Pseudomonas fluorescens (40.51%).

INTRODUCTION

Field bean (*Dolichos lablab*, L.) is one of the most ancient crops among cultivated plants and is presently grown throughout the tropics, especially in South Asia and African countries both pulse and vegetable crop (Rangaiah, 2016). This crop is known by several common names across the world, hyacinth bean (Brazil), Dolichos bean, Sem bean, Australian Pea (Australia), Kikuyu bean (Kenya), Gerenge (Ethiopia), Tonga bean (England), Lubia (Sudan), Fiwi bean (Zambia), Field bean, Indian bean, Country bean and Pole bean (India) *etc.*, (Aleksandar and Vesna, 2016) and different names in different parts of India-Telugu - Chikkudu, Tamil - Avarai, Marathi - Wal or Pavta, Hindi - Sem, Gujarati -Wal, Wal - papdi or Valor, Bengali - Shim, Malayalam - Mocca, Kanada - Chapparadaavare and Mai, Avaraa etc.,(Shivashankar and Kulkarni, 1989).

The crop is believed to have originated in India as documented by archaeo-botanical finds from 2000 to 1700 BC at Hallur, the earliest Iron-Age site in Karnataka from 1200 to 300 BC at Veerapuram excavation site in Andhra Pradesh (Fuller, 2003).

It is herbaceous multipurpose legume crop and used as animal feeding, in the form of fresh forage, hay, forage meal, grain and straw, grazing and browsing, and for human consumption, as fresh leaves, immature pods, immature grains and mature grains (Mihailovic *et al*., 2010). It is also used for green manuring, erosion control, nitrogen fixation and drought tolerance, cover crop in orchards and also as a weed smothering crop (Murphy and Colucci, 1999).

Field bean (Lablab purpureus) named as 'poor man's bean (Ismunadji and Arsyad, 1990). In spite of being important crop, it suffers many diseases starting from seed germination to crop maturity. In a worldwide survey involving the diseases of hyacinth bean, Colletotrichum lindemuthianum, Curvularia lunata (Wakker) Boedijn and Helminthosporium hyacinth beanis Sawada and Katsuki were reported by Duke *et al*. (1981). The major diseases that are threatening field bean production includes *viz*., anthracnose (Colletotrichum lindemuthianum), angular leaf spot (Phaeoisariopsis griseola), early blight (Alternaria solani), leaf spot (Cercospora dolichi), root and stem rot or ashy stem blight (Macrophomina phaseolina), powdery mildew (Leveillula taurica var. macrospora), rust (Uromyces appendiculatus), scab (Elsinoe canavaliae), bacterial leaf spot (Xanthomonas phaseoli), Dolichos yellow mosaic disease, dolichos venation mosaic and root knot nematode (Meloidogyne incognita) are reported by Narayanan and Dabadghao, (1972), Kay (1979), Shivashankar *et al*. (1987), Ismunadji and Arsyad (1990) and Yawalkar and Harihar Ram (2004) in India.

Schwartz and Galvez (1980) reported more than 50 per cent of major bean diseases are seed borne. Seed borne diseases affect seed germination, initial stand establishment, by causing abortion, shrinking, rotting, necrosis, discolouration, and weaker vigour of seeds, and carrying over of infection

across seasons ultimately affecting the yield (Shetty, 1992). Among the fungal diseases, anthracnose caused by Colletotrichum lindemuthianum (Sacc. and Magn.) Bri and Cav. is an important cosmopolitan, seed borne disease in humid and cool environments of the world and infects different plant parts like leaf, stem, pod and seed, and caused significant yield loss both qualitatively and quantitatively (Zaumeyer and Thomas, 1957). Zate *et al*. (1976) reported the seed borne infection intensity of C. lindemuthianum to the extent of 10 to 30% and Lakshmi Ramakrishnan (1964) reported seed borne infection (13.40%) due to C. lindemuthianum in Dolichos lablab. Anthracnose pathogen, can cause losses up to 100 per cent, if contaminated seed is planted and prolonged favourable conditions to disease development during the crop cycle in different parts of the world (Schwartz *et al*., 2005) as well as in India (Sharma *et al*., 2008).

Although the crop losses due to anthracnose can be reduce by application of fungicides, botanical and biological methods. Chemical control had been sought as the most effective measure to control the spread of disease and results in combating disease appears in the short period of time. Availability of new fungicides as well as in combination fungicides necessitates evaluation under in vitro conditions to know their efficacy before apply them in field conditions. Botanicals are biodegradable and their use in crop protection is a practical sustainable alternative to chemical methods of disease control and is unique because they can be produced easily by farmers and small scale industries. Similarly bio-agents are also becoming popular in farming community as they are eco friendly. Hence in the present study was initiated to find out the most effective fungicide(s), bio agent(s), plant extract(s) against spore germination of C. lindemuthianum under in vitro conditions.

MATERIALS AND METHODS

The sensitivity of C

lindemuthianum was tested in vitro by using three systemic fungicides *viz*., thiophanate methyl, propiconazole and azoxystrobin,two combi-fungicides namely captan + hexaconazole and carbendazim + mancozeb belonging to different chemical groups, two plant extracts *i.e*. Azadirachta indica and Lantana camara and three bioagents *viz*., Trichoderma viride, Pseudomonas fluorescens and Bacillus subtilis at Division of Plant Pathology, College of Horticulture,Dr.YSRHorticultural University, Venkataram annagudem,Andhra Pradesh.Each fungicide (0.05, 0.10 and 0.15%) and plant extract (5.0, 7.5 and 10.0%) were tested at three different concentrations, while one drop of cultural filtrate of bioagents was studied on spore germination of C. lindemuthianum by slide germination technique.

Preparation of plant extracts

Fresh healthy, disease free leaves of A. indica and L. camara were collected from field bunds of medicinal and aromatic block of Horticultural Research Station, V.R. Gudem. Plant extracts were prepared as per the procedure described by Jaganathan and Narsimhan (1988).

Preparation of culture filtrates of bioagents

Bioagents were grown individually on 100 ml of sterilized liquid media (nutrient broth for bacteria and potato dextrose broth for fungi) for production of culture filtrates in 250 ml conical flasks. The culture filtrates were obtained after 72 hr of incubation by filtering through five mm Whatmann No. 42 filter paper and then evaluated for spore germination studies.

In vitro studies of fungicides and plant extracts on spore germination.

The conidia of C. lindemuthianum were taken from 15 days old culture and conidial suspension was made separately for each concentration of different fungicides and plant extracts. Ten μl of each fungicide solution and plant extract suspension and ten μl of conidial suspension were mixed separately and the mixtures were transferred to surface of dried cavity slides and incubated at 25 ± 1ºC for 24 h in moisture chamber.

In vitro studies of culture filtrates of bioagents on spore germination.

One drop of pathogen spore suspension was placed at the centre of clean glass slide and mixed with one drop of bio-agent suspension using micropipette on different glass slides. The glass slides containing drop of mixed suspension was placed in an inverted position supported over two pieces of glass rods kept in a sterilized Petri plate lined with double-layered moist filter paper at 25 ± 1°C for 24 h in moisture chamber.

These experiments were laid out in completely randomized design with three, five and four replications for each treatment in case of fungicides, plant extracts and bioagents, respectively. A control set was also run concurrently in which spores were mixed in sterilized distilled water. After 48 hr, spore germination were recorded under the high power (40X) magnification of binocular microscope by adding one drop of lacto phenol cotton blue on the slides containing conidial suspension. Germination of conidia was defined as the germ tube presenting a longer/larger or equal size to the smallest conidial size (Tuite, 1969) and per cent spore germination was calculated by the following formulae:

$$\text{Percent spore germination} = \frac{A}{B} \times 100$$

Where,

A = Number of spores germinated

B = Number of spores observed

Further inhibition of spore germination was calculated as per the formula given by Vincent (1947).

$$I = \frac{(C - T}{C} \times 100$$

Where,

I = Per cent inhibition of spore germination

C = Spore germination in control

T = Spore germination in treatment

RESULTS AND DISCUSSION

In vitro efficacy of fungicides on spore germination

The tested fungicides *viz*., propiconazole, thiophanate methyl, carbendazim + mancozeb, captan + hexaconazole and azoxystrobin were numerically superior over the control in inhibiting the spore germination. The data presented in Table 1 showed that fungicides and concentrations were significant, while their interactions were non significant. Spore germination of test pathogen decreased with increase in their concentrations. Similarly, per cent inhibition of spore germination increased with the increasing concentration from 0.05 to 0.15 per cent of all the fungicides. The tested fungicides (each @ 0.05, 0.1 and 0.15%) inhibited spore germination in the range of 52.57 to 74.84% at 0.05% concentration, while it was ranged from 57.28 to 79.70 % and 66.50 to 85.44% at 0.1% and 0.15% concentrations, respectively, of C. lindemuthianum, over untreated control (Fig 1).

The mean per cent spore germination of C. lindemuthianum at three different concentrations showed the significant difference and ranged from 33.86 to 43.00. The highest mean spore germination (41.29%) was observed at 0.05 per cent concentration and the lowest mean per cent spore germination (35.27) was noticed at 0.15 per cent which was statistically at par with 0.1 per cent concentration (36.98).

The mean per cent spore germination with different fungicides ranged from 17.51 to 35.58. The per cent spore germination (24.48) with the corresponding spore inhibition (63.43) was noticed in propiconazole and was significantly superior to the rest of fungicides. This was followed by thiophanate methyl on spore germination and per cent reduction in spore germination, 28.50 and 58.82%, respectively. Fungicides *viz*., carbendazim + mancozeb (32.78%), captan + hexaconazole (35.74 %) and azoxystrobin (36.49%) were on par with each other on spore germination (Table 1).

The interaction of effect of fungicides and their different concentrations were non significant on spore germination and reduced the spore germination ranged from 12.93 to 42.30. Among the different fungicides tested, propiconazole was numerically superior over the rest of fungicides with lowest spore germination (21.03%) at 0.15 per cent concentration. It was evident from above the results that, irrespective of concentration and fungicides tested, propiconazole and thiophanate methyl were effective at all the concentrations in suppression of spore germination by interfering in the morphogenetic pathway of C. lindemuthianum.

The effect of different fungicides on spore germination of C. lindemuthianum was inhibited to greater extent by propiconazole followed by thiophanate methyl. Propiconazole is a triazole group of fungicide that has protective, curative and systemic activity and, it inhibit one specific enzyme, C14-demethylase [demethylation inhibitors (DMI's)], which plays a role in sterol production. Propiconazole blocks the demethylation of an intermediate compound in the synthesis of ergosterol from lanosterol. The production of ergosterol is interrupted, hence fungal cell membranes structure and function is affected.

Thiophanate methyl belongs to benzimidazole compound used as systemic foliar fungicide. The benzimidazoles interrupt the mitotic process by the specific binding of the active agent, carbendazim, to the tubulin subunits of the fungal cell resulting in a reduced rate of growth.

These results are in accordance with de Souza Filho *et al*. (2015) who reported that thiophanate methyl was the most effective against C. lindemuthianum. Similar finding on antifungal activities of propiconazole and thiophanate methyl have been reported by Jairo *et al*. (2012) and Silue Nakpalo *et al*. (2017) against various species of Colletotrichum and Kumar and Mauriya (2015) against Exserohilum turcicum.

In vitro bio efficacy of plant extracts on spore germination

The antimicrobial effect of two plant extracts on spore germination were tested at three different concentration levels *i.e.* 5.0, 7.5 and 10.0 % and data on spore germination of C. lindemuthianum are presented in Table 2. Though complete inhibition of spore germination was not observed with the plant extracts tested but considerable amount of reduction was noticed over the control. Data revealed that plant extracts tested numerically inhibited spore germination in range of 39.50 to 63.72% at 5.0% concentration, 55.18 to 70.92% at 7.5% concentration and 60.28 to 75.53% at 10.0% concentration over untreated control (Fig-2). Table 2 showed that botanicals, concentrations and their interactions were significantly effective on spore germination of C. lindemuthianum.

The mean spore germination of C. lindemuthianum at three different levels of concentrations showed the significant difference among them. The mean minimum spore germination was observed at 10.0 per cent (45.16%) and highest per cent spore germination (49.97%) at 5.0% concentration.

Mean per cent spore germination recorded with plant extracts ranged from 26.59 to 42.96 and they were significantly different. The lowest per cent spore germination (30.94%) was recorded with L. camara and highest per cent conidial germination (40.91) noticed in A. indica.

The interaction effect of plant extracts and their different concentrations were also found significant and it was ranged from 21.89 to 52.78. The per cent spore germination was recorded with L. camara (27.81) at 10.0% concentration, which was statistically on par with 7.5% concentration of L. camara (30.88). The per cent spore germination at 5.0 per cent concentration of L. camara (34.14) was statistically at par with A. indica at 10.0 and 7.5 per cent concentration (36.51 and 36.92), respectively. The highest spore germination (46.60%) was recorded at 5.0 per cent concentration of A. indica (Table 2). This study indicates L. camara at 5.0 per cent was equally effective with 7.5 and 10.0 per cent concentration of A. indica.

The effect of leaf extracts on inhibition spore germination of C. lindemuthianum to greater extent by L. camara, due to the presence of antimicrobial compounds such as phenolic compounds, flavonoids, saponins, tannins, phlobatanins, glycosides and alkaloids followed by A. indica due to azadirachtin, which retarded the spore germination. The present findings are agreement with the findings of Onifeda (2000) who observed inhibition of conidial germination of

Table 1: In vitro effect of fungicides on spore germination (%) of C. lindemuthianum.

Fungicides	Per cent spore germination *			Mean
	0.05%	0.10%	0.15%	
Propiconazole	22.71(28.31)**	16.88(24.11)**	12.93(21.03)**	17.51(24.48)**
Thiophanate methyl	28.54(32.23)	22.58(28.25)	18.11(25.02)	23.47(28.5)
Azoxystrobin	42.3(40.51)	34.78(36.06)	29.65(32.91)	35.58(36.49)
Carbendazim + mancozeb	34.94(36.13)	29.08(32.56)	24.65(29.65)	29.55(32.78)
Captan + hexaconazole	39.94(39.12)	33.97(35.58)	29.05(32.52)	34.32(35.74)
Control	89.57(71.45)	82.41(65.34)	88.74(70.51)	86.91(68.1)
Mean	43(41.29)	36.62(36.98)	33.86(35.27)	-
Factors	CD at 1%	SE (m) ±	SE (d)	
Concentrations (A)	2.71	1.33	0.94	
Fungicides (B)	3.83	1.88	1.33	
Interactions (AXB)	NS	3.23	2.3	

* Mean of three replications **Figures in parentheses are transformed (angular) values.

Table 2: In vitro effect of plant extracts on spore germination (%) of C. lindemuthianum.

Treatments	Per cent spore germination *			Mean
	5.00%	7.50%	10.00%	
Azadirachta indica	52.78(46.60)**	40.69(36.92)**	35.42(36.51)**	42.96(40.91)**
Lantana camara	31.51(34.14)	26.38(30.88)	21.89(27.81)	26.59(30.94)
Control	87.17(69.18)	90(72.6)	89.37(71.75)	89.15(70.97)
Mean	57.16(49.97)	52.64(47.7)	48.89(45.16)	-
Factors	CD at 1%	SE (m) ±	SE (d)	
Concentrations (A)	1.83	0.9	0.64	
Plant extracts (B)	1.83	0.9	0.64	
Interactions (AxB)	3.17	1.56	1.1	

* Mean of five replications **Figures in parentheses are transformed (angular) values.

Table 3: Effect of cultural filtrates of bioagents on spore germination (%) and spore inhibition (%) of C. lindemuthianum

S.No	Bioagents	Per cent spore germination *	Per cent inhibition of spore germination *
1	Trichoderma viride	40.66	53.51
2	Pseudomonas fluorescens	52.13	40.51
3	Bacillus subtilis	42.56	51.16
4	Control	87.42	-
	CD at 1%	5.33	
	SE(m) ±	1.64	
	CV (%)	6.72	

* Mean of four replications

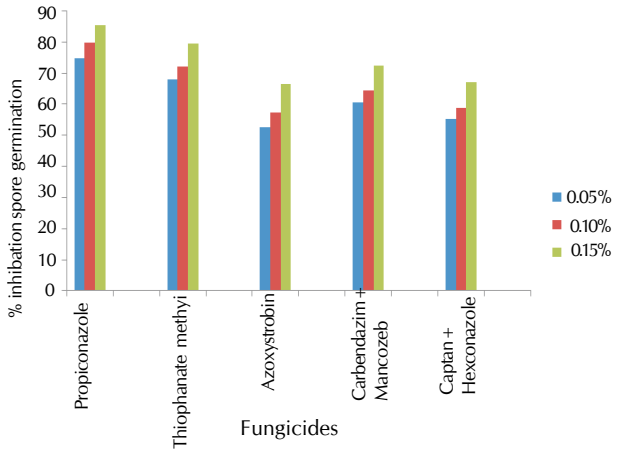

Figure.1 Effect of fungicides on per cent inhibition spore germination of C. lindemuthianum.

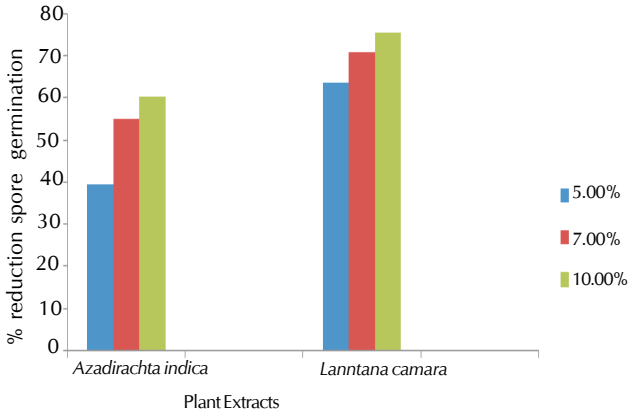

Figure 2: Effect of plant extracts on per cent inhibition spore germination of C. lindemuthianum.

C. lindemuthianum. These results further supported by findings of Ademe *et al*. (2013) who reported the ethyl acetate extracts of L. camara inhibited the highest spore germination (88.70%) followed by Lantana viburnoides (85.80%). And also Alam *et al*. (2002) who reported the bark extracts of A. indica at 25% concentration completely inhibited the conidial germination

of C. gloeosporioides. Similar finding on antimicrobial effects of L. camara and A. indica leaf extracts have been reported by Hedge *et al*. (2014), Rani *et al*. (2016) against different species of Colletotrichum and Singh and Dutta (2017) on Exserohilum turcicum.

Effect of cultural filtrates of antagonists on spore germination

The filter sterilized cultural filtrates of bioagents were tested against spore germination and results were presented in Table 3. The mean spore germination in the presence of cultural filtrates ranged from 40.66 to 52.13 per cent. Of the three bioagents tested, lowest spore germination (40.66%) coupled with highest inhibition of conidial germination (53.51%) was occurred in T. viride cultural filtrates and was significant superiority over P. fluorescens (52.13 and 40.51%). Whereas B. subtilis was moderately effective against conidial germination (42.56%) and inhibited the spore germination (51.16%) and statistically at par with spore germination and spore inhibition of T. viride.

The results of antagonistic activity of cultural filtrates of bioagents *viz*., T. viride, B. subtilis and P. fluorescens on spore germination due to production of volatile and non volatile compounds. These findings were in accordance with Manjunath *et al*. (2013), Parthiban and Kavitha (2014) against C. lindemuthianum. Similar kind of results were reported by Jairo *et al*. (2012), Azad *et al*. (2013) and Vivekanand et al. (2018) who quoted the T. viride, P. fluorescens and B. subtilis were efficient in inhibiting the spore germination of C. gloeosporioides and C. capsici, respectively, Singh and Singh (2014) against Exserohilum turcicum.

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NAAS Rating : 5.26

U.S.A. Office
2827 Videre Dr.,
Wilmington,
DE 19808 We, USA

Type seter Bandana Solutions Facility Management LLP
Published by Aditi Publications, Patliputra, Patna

The Bioscan
An International Quarterly Journal of Life Sciences
ISSN : 0973-7049
Volume 15(3) : 2020
Published as an official organ by
NATIONAL ENVIRONMENTALISTS ASSOCIATION

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AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

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