Annals of Plant and Soil Research 26(4): 605-610 (2024)
https://doi.org/10.47815/apsr.2024.10406

Collection and isolation of biocontrol agents to test the comparative efficacy against *Fusarium oxysporum* Schlecht causing wilt of Isabgol *In vitro*

SHAIK MUNNYSHA[1]*, BUNKER R.N.[1] AND MEGHA CHATURVEDI[2]

[1]Department of Plant Pathology, Maharana Pratap University of Agriculture and Technology, Udaipur-313001 (Raj.), India

Received, August, 2024; Revised accepted, November, 2024

ABSTRACT

Wilt of Isabgol caused by *Fusarium oxysporum* Schlecht is one of the most economically crucial diseases of Isabgol. The disease causes signficant yield losses over isabgol growing regions of the world. Although the injudicious use of chemicals is difficult to manage the wilt due to its persistance nature led to negative impact on beneficial microbiota in soil and harnessing the source of environmental pollution. Therefore, the present studies were emphasized with the collection and isolation of 10 different biocontrol agents from manufacture companies or pesticide dealers marketed at various locations of India. Notably, the recoveries of biocontrol agents were employed for suppression of *F. oxysporum in vitro* by using dual culture method. Among 10 different bioagents were assessed, *T. viride* (T.v-05) was found to be more effective against *F. oxysporum* with a maximum and significant per cent growth inhibition of 89.41.

Keywords: Isabgol, *Fusarium oxysporum* Schlecht, beneficial microbiota, *Trichoderma viride*

INTRODUCTION

Isabgol (*Plantago ovata* Forsk.) is a short stemmed medicinal annual herb belongs to *plantaginaceae* family which also referred as ‘Psyllium’ in English. It is native to Western Asia and Mediterranean regions stretches up to Sutlaj and Sindh in Pakistan. Isabgol grows up to a height of 35 to 40 cm. Isabgol is one of the most crucial crops among the cultivated medicinal plants grown for husk and seeds (Aishwath and Ram, 2008). The seed husk consisting of colloidal mucilage that crucially holding a good source of arabinose, xylose and galacturonic acid with rhamnose and galactose (Jat *et al*, 2015). Psyllium husk is chiefly used as regular herbal medicine to treat constipation and other digestive system disorders (Voderholzer *et al.,* 1997). In India, area under Isabgol cultivation was estimated at 448 thousand hectares with production of 432 thousand metric tonnes (Anonymous, 2018-19). India has exported 200.89 thousand lakhs of medicinal and aromatic plants (Anonymous, 2019-20). In India, *Plantago ovata* is commercially grown in the western states of Gujarat, Madhya Pradesh and Rajasthan during winter season. Currently, Isabgol has gained the name “Dollar earner” in north Gujarat and south western Rajasthan. In Rajasthan Isabgol is cultivated in about 3.49 lakh hectare with a production of 1.96 lakh tonnes and productivity about 563 kg per hectare (Agriculture statistics at a glance, 2020-21). In India, Isabgol is commercially grown in North Gujarat, Rajasthan, Madhya Pradesh, Haryana and Punjab (Rathore and Pathak, 2002). The major Isabgol growing districts in Rajasthan include Chittorgarh, Barmer, Jalore, Jodhpur, Pali, Sirohi and Udaipur.

Fusarium oxysporum Schlecht has deems crucial from phytopathological point of view as it is most serious soil-borne disease (Russel, 1975; Mehta *et al.*1985). Meena & Roy (2020) elucidated that Fusarium wilt is very crucial and highly devastating disease of Isabgol in Rajasthan which poses yield losses 20-40% causing significant damage in copious number of growing countries. The disease incidence is accomplished by several biotic and abiotic factors, with prevalence ranging from 10 to 60% (Meena and Satyajit, 2020). Similarly, Abhinav (2022) assessed copious number of genotypes for source of resistance against *Fusarium oxysporum* Schlecht causing wilt of isabgol. Out of nine genotypes screened, only five genotypes UI-124, UI-125, UI-6-1, UI-3-1

[2]Department of Entomology, Bundelkhand University, Jhansi-284128 (UP.), India
**Corresponding author: munnysha118@gmail.com*

and UI-2-1 were moderately resistant. In present study released elite varieties and genotypes were evaluated in field under sick plot method for two consecutive years. *Trichoderma* is predominant culturable fungi belonging to the ascomycete genus *Hypocrea*. *Trichoderma* spp. are multifunctional biological means for plant disease management (Cook and Baker, 1983). *Trichoderma* spp. is extensively protracted mixed mode of action *viz*., mycoparasitism, competition and antibiosis for the management of many plant pathogens and associated diseases (Bora and Rahman, 2022). For instance, *Trichoderma* and *Bacillus* has multifacated activity for suppressing fungal and bacterial diseases of plants (Bora *et al.,* 2022, 2023, 2024; Handique *et al.,* 2024). Nonetheless, promising bioformulation with integration of organic amendments along with the fungicides have been found effective to suppress the diseases and also improve host plant resistance (Kumar and Singh, 2017; Jagadish *et al.,* 2018; Choudhary *et al.,* 2017, Srivastava *et al.,* 2022).

The global biopesticides market is booming with a significant share of various commercial formulations of *Trichoderma* spp. However, the cost of these raw substrates or commercial production of bio-control agents is one of the major hurdles behind the restricted use. To overcome the cost limitation, many researchers have successfully employed copious number of agricultural wastes such as wheat bran-saw dust medium, wheat bran, biogas manure, farm yard manure, rice bran, peat soil, tea waste, rice straw corn fiber dry mass, vegetable waste, sewage sludge and compost to develop an effective formulation and biomass production of *Trichoderma* sp. (Mukhopadhyay *et al.* 1986; Srivastava *et al.,* 2016). Injudicious uses of pesticides have posed several negative impacts on the environment and human health. They also degrade soil fertility, build up resistance on pathogens, lead to failures in controlling disease. Emphasize should be given towards organic fertilizers and pesticides to achieve sustainability in agriculture. The *Trichoderma* is elucidated as microbial biocontrol agent against wide range of fungi that cause foliar, soil borne and root rot diseases. Although the promising biocontrol agents act as a substitute to commercial pesticides (Nikita and Chauhan, 2020).

MATERIALS AND METHODS

Collection and isolation of different bioagents and their comparative potential

Ten different biocontrol agents were procured and isolated from samples of different manufacture companies or pesticide dealers marketed at various locations of India (Table 1). Some bio-agents were collected from Bio-pesticide laboratory, Department of Entomology and Plant Pathology, MPUA&T-Udaipur.

For isolation of fungal strains, soil dilution plate method was employed. Soil suspensions were prepared by adding 0.5 g of soil in 50 ml of distilled water and keep it on orbital shaker for 10-15 minutes. Then after each suspension is serially diluted up to 10^3, pipette out 0.1 ml of sample from diluted soution and spread it on *Trichoderma* Selective Medium (TSM) and Potato Dextrose Agar (PDA) with the help of spreader under aseptic condition and incubate at 28°C for 7 days. The number of fungal colonies on the PDA and TSM plates was counted, and the total CFU/g of soil was calculated. The *Trichoderma* colonies were purified and stored at 4°C for further studies (Askew and Laing, 1993). Per cent growth inhibition of *F. oxysporum* by different bioagents was measured by using standard formula given by Vincent (1927).

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

Where,

I = Per cent growth inhibition zone of pathogen
C = Growth of test fungus in control (mm)
T = Growth of test fungus in treatment in dual culture (mm)

The biological control ability of different biocontrol agents were determined using the Pakdaman's biological control index (PBCI) with following parameters:

Pathogen resistance index (R) = Full growth of BCA in presence of pathogen (Z) /Full growth of BCA in absence of pathogen (M)

$$\text{PBCI} = \frac{\text{M (in days)}}{\text{Z (in days)} \times \text{P (in mm)}}$$

Where,

C= Days required for the two colonies to come into contact
Z= Days required for the BCA to fully grow over the pathogen colony
M= Days required for the BCA to fully grow over the plate
P= The radial growth distance (in mm) of pathogen colony between the point of inoculation and the marginal point of contact with BCA growth zone

Evaluation of different bioagents against *F. oxysporum* Schlecht *in-vitro*:

Table 1: List of different bioagents against *F. oxysporum* Schlecht i*n-vitro*

Designation	Name of the bioagent	Procured place
T.v-01	*T. viride*	Isolated from chilli crop, RCA, Horticulture farm, MPUA&T-Udaipur
T.v-02	*T. viride*	Gujarat Eco Microbial Technologies Private Ltd. Gujarat
T.v-03	*T. viride*	PCI- Pest Control India, Private LTD, Bangalore
T.v-04	*T. viride*	IARI, New Delhi
T.v-05	*T. viride*	Bio-pesticide laboratory, Department of Entomology, MPUA&T-Udaipur
T.h-01	*T. harzianum*	Jhadol, Udaipur
T.h-02	*T. harzianum*	Isolated from maize crop, Udaipur
T.a-01	*T. aureoviride*	Dungarpur
M.a-01	*Metarhizium anisopliae*	Indian Agri Farm, Tamil Nadu
P.l-01	*Paecilomyces lilacinus*	Indian Agri Farm, Tamil Nadu

Results

In-vitro evaluation of biocontrol agents by dual culture technique

The comparative efficacies of different bioagents were evaluated against *Fusarium oxysporum in vitro* by dual culture method as presented in the material and methods. Among 10 different bioagents were assessed, *T. viride* (T.v-05) was found to be more effective against *F. oxysporum* with a maximum and significant per cent growth inhibition of 89.41. The other bioagents viz., *T. viride* (T.v-03), *Paecilomyces lilacinus* (P.l-01), *T. harzianum* (T.h-01), *Metarhizium anisopliae* (M.a-01), *T. viride* (T.v-02), *T. harzianum* (T.h-02), *T. viride* (T.v-01), *T. aureoviride* (T.a-01) and *T. viride* (T.v-04) were next to the above mentioned isolates and exhibited 80.65, 79.37, 77.47, 70.21, 65.81, 60.74, 56.16, 38.98 and 36.16 per cent growth inhibition of *F. oxysporum* Schlecht respectively (Table 2).

Table 2: Comparative efficacy of bioagents against *Fusarium oxysporum* Schlecht *in vitro*

Sl. No.	Treatments	C (days)*	Z (days)	M (days)	P (mm)	PCBI	MGI (%)
1	*T. viride* (T.v.- 05)	3.25	5.25	4.25	13.25	0.060	89.41
2	*T. viride* (T.v.- 03)	3.25	5.25	4.25	14.75	0.055	80.65
3	*T. viride* (T.v.- 01)	3.75	5.75	4.25	17.75	0.042	56.16
4	*T. viride* (T.v.- 04)	3.75	5.75	4.25	26.75	0.027	36.16
5	*T. viride* (T.v.- 02)	3.25	5.25	4.75	20.25	0.044	65.81
6	*T. aureoviride* (T.a-01)	3.5	6.5	4.75	25.25	0.028	38.98
7	*T. harzianum* (T.h.-01)	3.25	5.75	4.5	22.25	0.035	77.47
8	*T. harzianum* (T.h.-02)	4	6.5	4.75	24.75	0.029	60.74
9	*Metarhizium anisopliae* (M.a-01)	3.25	5.25	4.25	19.25	0.041	70.21
10	*Paecilomyces lilacinus* (P.l.-01)	3.75	5.75	4.25	23.75	0.031	79.37
	SE±m	0.241	0.258	0.254	0.250	0.003	0.466
	CD at 5%	0.697	0.745	0.734	0.722	0.009	1.345

**Mean of four replications, Where, C= Days required for the two colonies to come into contact, Z= Days required for the BCA to fully grow over the pathogen colony, M= Days required for the BCA to fully grow over the plate, P= The radial growth distance (in mm) of pathogen colony between the point of inoculation and the marginal point of contact with BCA growth zone*

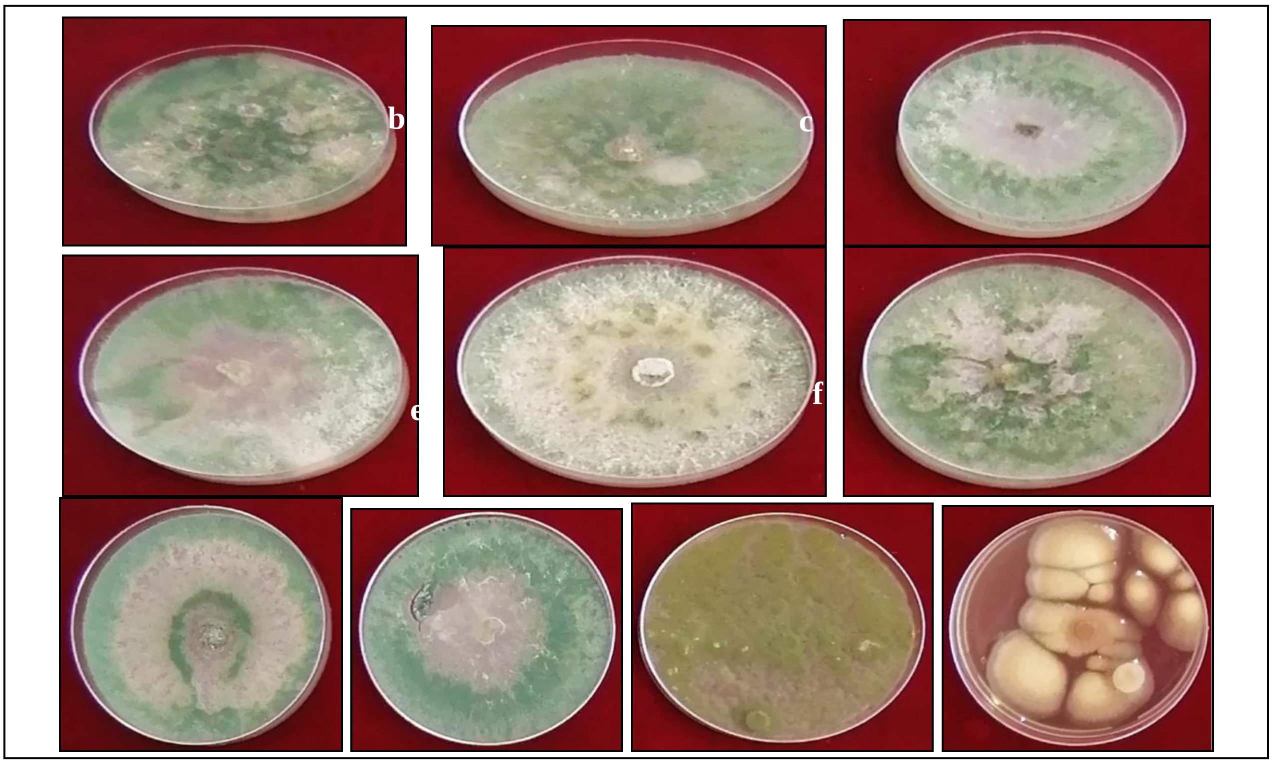

Plate 1: Different Bioagents used for present course of study

(1) *T. viride* (T.v-05); (2) *T. viride* (T.v-02); (3) *T. viride* (T.v-01); (4) *T. viride* (T.v-05); (5) *T. aureoviride* (T.a-01); (6) *T. viride* (T.v-03); (7) *T. harzianum* (T.h-01); (8) *T. harzianum* (T.h-02); (9) *Metarhizium anisopliae* (M.a-01); (10) *Paecilomyces lilacinus* (P.l-01)

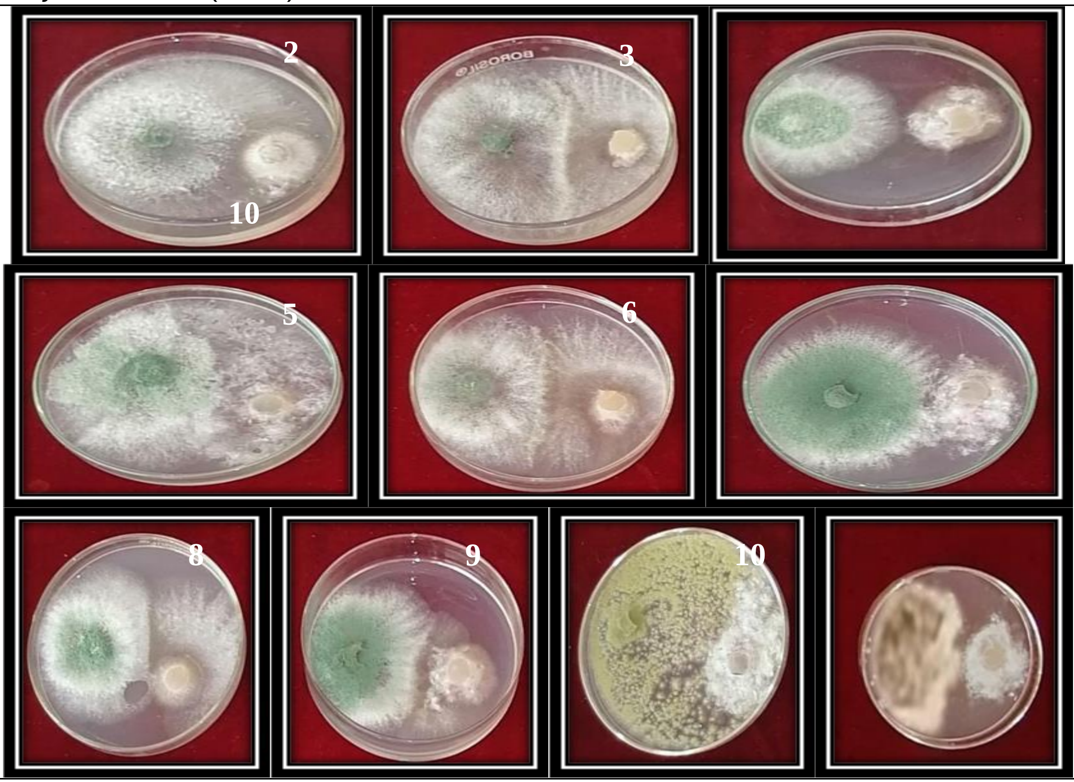

Plate 2: Efficacy of different bioagents against the growth of *F. oxysporum* Schlecht *in vitro*

(1) *T. viride* (T.v-05); (2) *T. viride* (T.v-02); (3) *T. viride* (T.v-01); (4) *T. viride* (T.v-05); (5) *T. aureoviride* (T.a-01); (6) *T. viride* (T.v-03); (7) *T. harzianum* (T.h-01); (8) *T. harzianum* (T.h-02); (9) *Metarhizium anisopliae* (M.a-01); (10) *Paecilomyces lilacinus* (P.l-01).

DISCUSSION

In order to achieve sustainable agriculture, chemicals must be eliminated, efficient biocontrol agents must encourage. The use of *Trichoderma* spp. are extensively used for suppression of fungal diseases of various agricultural crops. Thus, use of bio-agents, organic amendments along with the fungicides have been found effective to suppress the diseases and also improve host plant resistance (Kumar and Singh, 2017; Jagadish *et al.,* 2018; Choudhary *et al.,* 2017). Baker and Paulitz, 1996 elucidated that bioagents suppress the fungal pathogen with mode of action employed by mycoparasitism or antibiosis. Similarly, other researcher emphasized that *Trichoderma* spp. have antagonistically and biologically suppress a soil borne pathogen *F. oxysporum* (Hanson and Howell, 2004; Afzal *et al.,* 2013). A study by Bhale (2016), revealed that biomass production of *Trichoderma* species worldwide. For instance, *T. asperellum* and *T. harzianum* was fortified with different substrates for assessment of biomass production. Exploitation of fungal antagonists paving a crucial role in sustainable agricultural tactics while mitigating hazardous pesticides. Notably, the commercial application of these fungal antagonists totally depends on cost effective formulations employed significantly higher viability at the time of utilization with good coverage and retention after exploration in the field.

CONCLUSION AND FUTURE PROSPECTS

From the present investigation, it may be concluded that among the different bioagents were evaluated against *Fusarium oxysporum in vitro. Trichoderma viride* (T.v-05) has been proven more efficient against *F. oxysporum* with a maximum and significant per cent growth inhibition. However, the exploitation and demand of biopesticides increasing rapidly all over the world. Although biopesticides has led to safer and sustainable farming practices as substitute to other pesticides. Consequently, it is crucial for farmers should be made aware as well as encouraged to use biopesticides to uphold sustainable agriculture

ACKNOWLEDGEMENT

The authors express thanks to the Head, Department of Plant Pathology and Dean, Rajasthan College of Agriculture, Udaipur for providing for providing bioagents and valuable suggestions for present research work.

REFERENCES

Abhinav, (2022) Biology and Management of Isabgol (*Plantago ovata* Forsk) wilt caused by *Fusarium oxysporum* Schlecht. (Doctoral Thesis, RCA, MPUAT). http://hdl.handle.net/10603/510707

Agricultural Statistics, 2018-2019. Directorate of Economic & Statistics, Department of Planning. Tilak Marg, Jaipur, Rajasthan, 302005. https://agriwerfare.gov.in

Aishwath, O.P., Ram, C. (2008) Response of blond psyllium (*Plantago ovata*) to split application of nitrogen on growth, yield and quality. *Indian Journal of Agricultural Sciences*. 78: 323-327. https://www.researchgate.net/publication/297141459

Anonymous, 2018-2019. Indian Horticulture Database, National Horticulture Board, 112. https://www.nhb.gov.in/Statistics. aspx? Type=State&menu. Menu=12

Anonymous, 2019-2020. Directorate general of foreign trade, Ministry of Commerce & Industry, New Delhi. https://www.dgft.gov.in/

Afzal, S., Tariq, S., Sultana, V., Ara, J. and Ehtashamul, H.S. (2013) Management the root rot disease of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. *Pakistan Journal of Botany.***45**(4): 1455-1460.

Askew, D.J. and Laing, M.D. (1993) An adopted selective medium for the quantitative isolation on *Trichoderma* species. *Plant Pathology,* **42**: 686-690.

Baker, R. and Paulitz, T.C. (1996) Theoretical basis for microbial interaction leading to biological control of soil borne plant pathogens. In: Principles and practices of managing soil borne plant pathogens. (Eds.): R. Hall. St. Paul, MN, APS Press. pp. 50-79.

Bhale, U.N. (2016) Prospective of agricultural wastes as base resources for mass multiplication of *Trichoderma* species worldwide: an overview. *International Jour. of Current Research*, 8:2496824978

Bora, P. and Rahman, M. (2022) Microbes Knocking Louder Roles for Next Green Revolution: A Meta-Analysis. *Annals of Plant and Soil Res,* 24(4):552-563 (2022) https://doi.org/10.47815/apsr.2022.10208

Bora, P., Gogoi, S., Deshpande, M.V. (2023) Rhizospheric *Bacillus* spp. exhibit miticidal efficacy against oligonychus coffeae (Acari: tetranychidae) of tea. *Microorganisms,* 11: 2691 https://doi.org/10.3390/microorganisms

Bora, P. Saikia, B., Rahman, M. Ahmed, S.S. Chetia, R. Rahman N. Nath, B.C, Raja, W.H. (2024) Enhancing the performance of chilli (*Capsicum annuum*) through twin role of plant growth promotion and disease suppressionvia *Bacillus subtilis*-based bioformulation. *The Indian Journal of Agricultural Sciences.* **94**(1):039-043.

Bora, P., Bora, L.C., Bhuyan, R.P., Hashem, A., Abd-Allah, E.F. (2022) Bioagent consortia assisted suppression in grey blight disease with enhanced leaf nutrients and biochemical properties of tea (*Camellia sinensis*). *Biological Control, 170*, 104907

Choudhary, A. Ashraf, S. and Musheer, N. (2017) Screening of phytoextracts to control of *Fusariumoxysporum* f.sp. *vigni* incitant of mungbean (*Vigna radiata*) wilt. *International Journal of Academic Res. and Development*, **2**(6): 1181-1184.

Cook, R.J. and Baker, K.F. (1983) The nature and practice of biological control of plant pathogens. *American Phytopathological Society*, p.539.

Handique, M., Bora, P., Ziogas, V.; Srivastava, A.K., Jagannadham, P.T.K., Das, A.K. (2024) *Phytophthora* Infection Reorients the Composition of Rhizospheric Microbial Assembly in Khasi Mandarin (*Citrus reticulata* Blanco). *Agro. 14*, 661. https://doi.org/10.3390/agronomy14040661

Hanson, L.E. and Howell, I. (2004) Elicitors of plant defence responses biological control strains of *Trichoderma virens*. *Phytopathology,* 94: 171-176.

Jagadish, S.G., Mallesh, S.B., Jaipraksah Narayan R. P. and Mallikarjun D. (2018) Screening of Bioagents against Wilt Complex Pathogens of Tomato. *International Journal of Pure and Applied Bioscie*nce, **6**(1):1486-1493.

Jat, R.S., Reddy, R.N., Bansal, S. and Manivel, P. (2015) Good agricultural practices for isabgol: *Extension Bulletin*. Boriavi, Anand: ICAR, *Directorate of Medicinal and Aromatic Plants Research*.

Kumar, N. and Singh, S.K. (2017) Screening of tolerance and compatibility of *Trichoderma viride* against common fertilizers and fungicides. *International Journal of Chemical Studies*, **5(4):** 1871-1874.

Meena, R.P., Roy, S. (2020) Morphological and molecular variability characterization of Fusarium sp. causing wilt disease of Isabgol (*Plantago ovata* Forsk.) and its management strategies. *Journal of Applied Research on Medicinal and Aromatic Plants*, **16**: 100244.

Meena R.P. and Satyajit, R. (2020) Morphological and molecular characterization of *Fusarium* spp. causing wilt of isabgol. *Jour. of App. Res. on Medicinal and Aromatic Pl,* **2**(44): 520-524.

Mehta, N, Madan, R.L., Thakur, D.P. (1985) Record of Isabgol wilt from Haryana. Haryana Agricultural University, *Journal of Agricultural Research*, 15, 473-474.

Mukhopadhya, A.N., Brahm, B.A. and Patel, G.J. (1986) *Trichoderma harzianum* - a potential biocontrol agent for tobacco damping off. *Tobacco Res,* 12: 26-35.

Nikita, B. and P.S. Chauhan (2020) Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress. DOI: 10.5772/intechopen.94593

Rathore, B.S. and Pathak, V.N. (2002) Studies on the tissue culture and host range of *Peronospora alta* causing downy mildew of blond psyllium. *Journal of Mycology and Plant Pathology,* **32**(2): 201-203.

Russel, TE. (1975) Plantago wilt. *Indian Phytopathology,* **65**: 359-360.

Srivastava, A.K., Das, A.K., Jagannadham, P.T.K., Bora, P., Ansari, F.A., Bhate, R. (2022) Bioprospecting microbiome for soil and plant health management amidst huanglongbing threat in citrus: A review. *Frontiers in Plant Science,* 13. doi: 10.3389/fpls.2022.858842

Srivastava, M., Kumar, V., Shahid, M., Pandey, S. and Singh, A. (2016) *Trichoderma*-a potential and effective bio fungicide and alternative source against notable phytopathogens. *African Journal of Agricultural Research,* **11**(5): 310-316.

Vincent, J.M. 1927) Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **15**: 850.

Voderholzer, W.A., Schatke, W. and Muhldonfer, B.E. (1997) Clinical response to dietary fibre treatment of chronic constipation. *American Journal of Gastroenterology*, **92**: 95-98.