ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Development and Evaluation of a Liquid Formulation of *Trichoderma viride* as a Bio-pesticide for Pest Management

Kalpesh Sorathiya[1*], Kuldip Sorathiya[1], Shreya Kalariya[1], Lensi Patel[1]

[1]Department of Research and development, Terrapreta Agrovet, Ahmedabad – 382210, India

Article Information

Received: 10 May 2023
Revised version received: 17 June 2023
Accepted: 20 June 2023
Published: 29 June 2023

Cite this article as:
K. Sorathiya et al. (2023) Int. J. Appl. Sci. Biotechnol. Vol 11(2): 60-65. DOI: 10.3126/ijasbt.v11i2.56119

***Corresponding author**
Kalpesh Sorathiya,
Department of Research and development, Terrapreta Agrovet, Ahmedabad – 382210, India
Email: kalpeshsorathiya02@gmail.com

Peer reviewed under authority of IJASBT

Abstract

The use of bio pesticides in modern agriculture is acknowledged as an efficient substitute for chemical pesticides because they are based on natural ingredients. Fungal bio pesticides are the most widely used and have a lot of success stories among the various bio pesticides. *Trichoderma* is a genus of filamentous fungi that has several promising bio control agents for different plant diseases. *Trichoderma viride* uses a variety of strategies to fight plant diseases, including high reproducibility, adaptability, production of enzymes and antibiotics, capacity to colonies the rhizosphere, and significant inhibitory effects against phytophathogenic fungi. The prospective *Trichoderma viride* isolates are produced utilizing various carriers either through solid or liquid fermentation technologies, however solid fermentation produces the highest levels of reproductive capacity. High cost of substrate and storge methods are major problems to accelerate the production. The aim of our research is commercially available substrates were carried out to screen out stabilization of formulations for small scale production of *Trichoderma vride*.

Keywords: Fungi; *Trichoderma viride*; Bio-pesticide; Formulation; Commercialization.

Introduction

In order to increase the production of food around the world, modern agriculture uses pesticides and chemical fertilizers. To meet the need for food demand, the plant grows quickly and effectively when Chemical fertilizers are used. The negatives of employing a bigger quantity of more chemical or synthetic fertilizers include environmental contamination, long-term changes in the ecology and physiochemistry of the soil, decreased agricultural productivity, and several health risks. Constant use of chemical pesticides degrades soil quality and fertility, increases the likelihood that heavy metals may accumulate in plant tissues, and reduces the nutritional content and edibility of fruit (Farina and Hasanpoor, 2015; Sneha *et al*., 2015). Climate factors increase abiotic stress on crops, reducing agricultural productivity. (Chaudhari *et al*., 2023). Over the years, agriculture has undergone numerous changes. Since then, a wide variety of organic fertilizers that act as organic stimulators for plant growth have been developed. A unique kind of organic fertilizers includes

products based on "bio-fertilizers," which are microorganisms that encourage plant growth. A material called bio-fertilizer contains biological microorganisms. When sprayed to plant surfaces, they promote plant development by supplying the host plant with more crucial nutrients. In addition to producing compounds that promote growth, bio-fertilizers provide nutrients through natural processes such nitrogen fixation, phosphorus solubilization, and stimulation of plant growth (Ajmal *et al*., 2018).

The health of the soil and plant productivity are significantly influenced by numerous interactions between plants, soil and microorganisms (Harman *et al*., 2020). The ecological balance of soil is maintained by soil microorganisms working in various ways with plant roots and one another to carry out a variety of vital activities (Kumar and Mohapatra, 2021). Since bio fertilizers support the natural soil micro biota, which affects nutrient accessibility and organic matter breakdown, they are a likely strategy to improve soil microbial status (Chaudhari *et al*., 2021). The potential of bio fertilizers to produce a high level of microbial diversity in soil may lead to more productive crops for sustainable agriculture (Agri *et al*., 2022).

The result will be a decreased demand for chemical fertilizers and assistance, while the significance of bio-pesticides is increasing. (Vaishali, 2014). Bio-pesticides are pesticides have gained a lot of interest from the scientific community, which has proposed they could replace synthetic pesticides manufactured from naturally occurring biochemical, microbes, and plants that are safe for the environment. Not all organic products function as bio pesticides. If they affect the pest's nervous system, some are chemical insecticides. Expanding the use of bio pesticides can help health programmes. Bio pesticides have been developed as a result of the problems that chemical pesticides cause, including genetic alterations in plant populations, a decline in beneficial species, harm to the environment or water bodies, food poisoning, and health problems like cancer. The risk of chemical exposure is reduced, fertilizer runoff causes less water pollution, and there are fewer applications, harms beneficial pests less, is biodegradable, and improves nutritional quality (Aneja *et al*., 2016).

Bio pesticides can be used to manage pests that harm plants. They can be living things (natural enemies), their byproducts (phytochemicals, microbial products). Both the environment and human health are less at risk from them. The organisms that are harmful to the target pest are the most frequently used bio pesticides includes bio pesticides (*Bacillus thuringiensis*) and herbicides (*Phytopthora*) (Opender, 2012).

Plant-harming pests can be controlled with the help of bio pesticides. Natural enemies are live entities, and their byproducts are either living things (phytochemicals, microbial compounds), or both. They pose less risk to the environment and human health. The most common bio pesticides are those that are detrimental to the target pest. Contains Phytopthora herbicides and *Bacillus thuringiensis* bio pesticides (Bhattacharjee and Dey 2014).

Similar to the use of chemical pesticides, microbial pathogens are disease-causing organisms that proliferate widely among pest populations. Through introduction or flooding applications, these pathogens are used for biological pest control. Like other living things, insects are susceptible to a number of diseases brought on by viruses, bacteria, fungus, protozoa, and nematodes, among other kinds of microorganisms. To create crop protection methods that are friendly to the environment, researchers have examined insect microbial infections in great detail. The development of chemical resistance and residues at higher nutrient levels is a significant barrier to pest control in the crop protection environment of today. To combat the negative impacts of chemicals on non-target organisms, bio pesticides have recently taken the place of chemical pesticides (Kachhawa, 2017).

Numerous species of the fungus genus *Trichoderma* are used as industrial bio pesticides (Kumar, 2017). *Trichoderma* is a fungus that grows quickly and is frequently used as a growth booster and biological control agent to combat soil-borne plant illnesses. Due to their various modes of action, filamentous fungi of the genus *Trichoderma* are among the best studied bio pesticides. Direct mycoparasitism, the creation of enzymes and antibiotics, competition, and the capacity to make plants tolerant to a variety of stressors are just a few of the stategies they use to manage plant pathogens (Alwadai, 2022).

The pathogen-suppression arsenal of *Trichoderma* species encompasses a variety (overparasitism), antibiotics, competition, and induced resistance (Sharmas, 2019) Competition between *Trichoderma* species and mycoparasites is frequently used to suppress soil-borne pathogens (Mukherjee *et al*., 2022). A putative *Trichoderma* species has been suggested by a recent study by Ren X *et al*. (2022). Inhibits the formation of aflatoxin B1 by isolates of aflatoxinogenic *Aspergillus flavus*. This implies that employing *Trichoderma* species as a bio control agent against *A. flavus* and preventing a flatoxin accumulation can be a successful bio control strategy, particularly when many isolates with various modes of action are combined (Ren *et al*., 2022).

Trichoderma sp. biomass and formulations must be produced in large quantities at low cost using locally accessible strains and inexpensive raw materials, such as diverse wastes and byproducts. With or without minor adjustments to the culture medium's composition, sufficient biomass yields, including effective propagules (such as

chlamydospores), can be achieved utilising such inexpensive growth substrates (Kumar *et al*., 2014).

Trichoderma viride is very capable of procreating and sporulating, as well as being competitive and surviving through saprophytic growth (Howell, 2013). *Trichoderma* strains expand quickly when injected into soil due to their innate resistance to a variety of harmful substances, such as pesticides, fungicides, and herbicides (Seethapathy *et al*., 2017). A range of trash and byproducts that can be employed as growth substrates to produce sufficient biomass containing effective propagules with little to no media composition adjustment. This review describes his two primary techniques for producing *Trichoderma* species in large quantities: liquid fermentation and solid-state fermentation (Kumhar *et al*., 2014).

It has enormous potential for commercial *Trichoderma* spp. bulk production. Finding alternatives to pesticides and chemical fertilizers to control widespread plant diseases and *Trichoderma* spp. plant growth stimulants has been the focus of research (Kumhar *et al*., 2014). There have been numerous research on the production of *Trichoderma* spp. utilising traditional synthetic media such glucose, cellulose, soluble starch, and molasses. In order for bio control products to be successful commercially,

- Consistent and widespread action.
- Security and Stability.
- Longer shelf life.
- Low capital cost.
- Ready Availability of Carrier Materials.
- Economic and viable market demands

This research provides information on effective approache s, such as biopesticides, to help rehabilitate agricultural soi ls and thereby promote sustainable growth (Glass *et al*., 2002).

Materials and Methods

Isolation and Identification of Trichoderma viride

There are a number of techniques for isolating *Trichoderma*, however, serial dilution of samples is one of the techniques that has been documented most frequently in the literature (Seethapathy *et al*., 2017; Glass *et al*., 2002; Hewavitharana *et al*., 2018). This method is easy, economical, and suitable for handling big samples. Take a *Trichoderma viride* sample that is commercially available in market and inject it into a maize plant. Take a soil sample from the soil of the maize plant after ten days. 10 ml of sterile, distilled water should be added to 1g of the soil sample before mixing well. Incubate the supernatant for 4 to 5 days at 25° to 30°C after spreading it over a potato dextrose agar plate. To obtain a pure culture, *Trichoderma* colonies were subculture on fresh PDA plates (Hewavitharana *et al*., 2018). Microscopic analysis is used to identify *Trichoderma viride.*

Mass Production Technology

A) Mass Production of Trichoderma viride in Liquid State Fermentation

Liquid state fermentation (LSF) is applied for the processes in which soluble materials in water is used for the microbial growth. Through the use of liquid fermentation technology, *Trichoderma* spp. are typically produced in large quantities using potato dextrose broth, molasses-yeast medium, and wheat bran (Srivastava *et al*., 2016).For the mass production black gram soak water, etc. are also employed.

Prepare 100ml of Potato dextrose broth and incubate it for 15 min at 121˚c temperature and 15psi pressure and cooled it room temperature before inoculation.Add 0.1gm antibiotic in broth for inhibition of bacterial growth and Inoculate 1ml of *Trichoderma viride* suspension in 100ml of Potato dextrose broth and then incubate at 25 ˚c to 30˚c for 5 to 6 days.

B) Mass production of Trichoderma viride in Solid state fermentation

For mass multiplication the selected medium should be inexpensive and readily available with appropriate nutrient balance. Solid-state fermentation (SSF) is an effective method for the mass production of fungal biopesticides since it provides micropropagules with higher conidia content. Various cheap cereal grains like, sorghum, rice, millets, ragi are used as substrates (Ramanu jam *et al*., 2010). For mass production of *Trichoderma viride* in sorghum take 100gm sorghum in 250ml flask and soak in 2% sucrose solution for 6 hour.After that remove water from flask though filtration and sterilized in autoclave.Cooled at room temperature and next day inoculated with 10ml of *Trichoderma viride* suspension and incubated for 10 to 15 days for production of *Trichoderma viride* which produces dark green spore coating on the grains which showed in Fig. 3. (Hewavitharana *et al*., 2018)

Optimization and Formulation in Liquid Carrier Material

Although *Trichoderma* has excellent potential for treating diseases, it could not be used in the field as a suspension of spores. To facilitate application, storage, commercialization, and field use, the *Trichoderma* culture should be produced as formulations and immobilized in certain carriers. *Trichoderma* spp. were cultured on sorghum for the final product's formulation. After the sorghum flasks have been incubated for 7 days, each one should be filled with distilled water before the liquid is extracted using filter paper. Then optimized for CFU count.

For optimization, several sugar solution concentrations of 0.1%, 1%, 10%, 15%, and 20% were created. 100 ml of an Erlenmeyer flask were filled with the prepared liquid carrier material. Prior to inoculation, all media were sterilized in an autoclave at 121 °C (15 psi) for 25 minutes and allowed to cool. Each flask inoculates with an antibiotic to prevent

bacterial development. *Trichoderma* viride liquid solution were inoculated into all liquid carrier material under sterile conditions and incubated at room temperature (28 ^{o}C) on a shaker at 60 rpm for 180 days. According to Kumar *et al*. (2014) CFU of *Trichoderma* should be a minimum of 2 x 10^6 CFU per ml or gram in selective medium of *Trichoderma*.Other microbial contaminants should not have more than 1 x 10^4 CFU/ml, and pathogenic contaminants should not be present. The viability of the Trichoderma formulations should be maintained for a considerable period of time.

Results

Isolation and Identification of Trichoderma viride

The fungal population isolated from soils and spread on the PDA plates. The dilution plate method was used for the isolation of pure fungi from soil samples. Identified pure colony from mix population by its colony characteristics and then spread on new PDA plates. *Trichoderma* isolates were identified microscopically which is showed in Fig. 1.

The fungal population was isolated from the soil and spread on the PDA plates. The dilution plate method was used for the isolation of pure fungi from soil samples. Identified a pure colony from a mixed population by its colony characteristics and then spread on new PDA plates. *Trichoderma* isolates were identified microscopically, as shown in Fig. 1. The evaluation of the growth performance of *Trichoderma viride* is shown in Fig. 2.

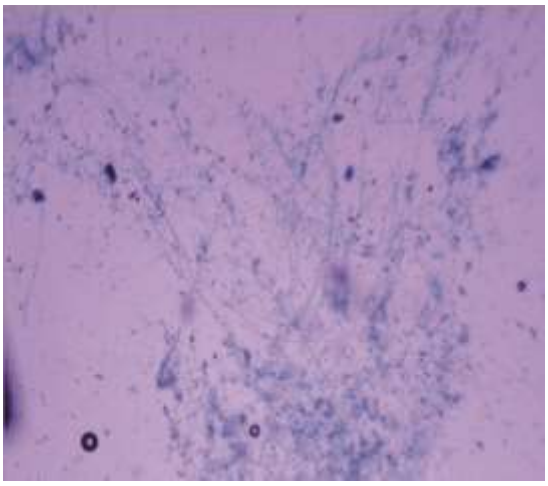

Fig. 1: Microscopic view of Trichoderma viride

Mass Production of Trichoderma viride in Liquid State Fermentation

The results of the liquid-state fermentation revealed that locally available liquid substrates such as coconut water, rice mill effluent, and black gram soak water are used for the mass production of *Trichoderma viride.* We are using potato dextrose broth for mass production of *Trichoderma viride*. It showed high sporulation. A study revealed that sporulation of T. viride is high in liquid media compared to solid media of the same substrate. *Trichoderma* is grown in the liquid medium, which is mixed with demineralized water for formulation with respect to the number of CFU counts.

Fig. 2: Evalution of the growth performance of *Trichoderma viride*

Fig. 3: Growth performance of *Trichoderma viride* in sorghum

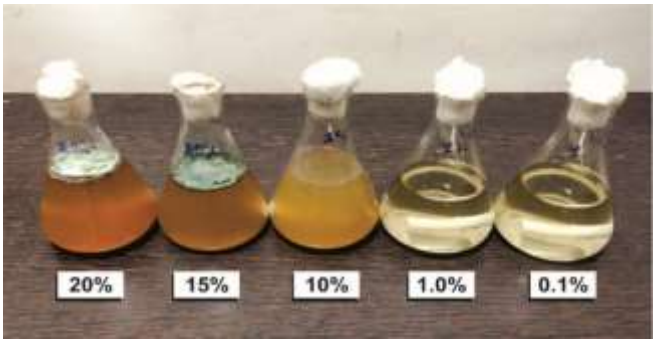

Fig. 4: Optimization of *Trichoderma* in sugar solution

Mass Production of Trichoderma viride in Solid State Fermentation

There was a significant difference in the growth of *T. viride* in different solid carrier materials with respect to the number of CFU/ml produced in the selected solid carrier materials. Following the CFU/ml values, the highest stabilization potential was recorded for sorghum after 9 days of fermentation for *Trichoderma* viride. At the end of the fermentation period, the conidia in the medium are harvested. A common way of separating the conidia from the medium is by filtration or centrifugation. Active component by combining it with other active (functional) and non-active (inert) ingredients. Water, oil, polymer, or a combination of these can all be used in liquid formulations (Grewal, 2005). Demineralized water and a 0.1% sugar-based solution are used as the carrier material in the current investigation. This formulation was determined to be acceptable for *Trichoderma* spp. growth and viability during storage. Up to 120 days, viability and survival were assessed. After 120 days, *Trichoderma's* maximum growth and higher survival stabilization are subject to change. The inoculant must have at least 2×10^6 CFU/ml. The colony forming units of the concentrated *Trichoderma viride* solution that were obtained after filtering were displayed in Table 1.

Table 1: The colony-forming units (CFU) of *Trichoderma viride*

No.	Dilution	No. of colonies	cfu/ml
1	10^{-1}	Lown growth	-
2	10^{-2}	35	3×10^4
3	10^{-3}	20	2×10^5
4	10^{-4}	8	8×10^5
5	10^{-5}	6	6×10^6
6	10^{-6}	4	4×10^7

Discussion

In agriculture, fungi *Trichoderma viride* has been widely used in agriculture as a biopesticide because of its ability to protect against phytopathogens. Our results revealed that Trichoderma fungi have good potential for mass production on locally available substrates in liquid as well as solid-state fermentation. A study reported that the growth performance of *Trichoderma viride* on PDA showed maximum at 192 hours of incubation period.

In liquid state fermentation growth of *Trichodrma viride* on potato dextrose broth showed significant growth at 6th day of incubation. It is a popular method for producing large quantities. The choice of liquid medium is crucial for the growth of *Trichoderma viride.*

When using sorghum for the growth of *Trichoderma* in solid-state fermentation (SSF), the moisture content of the sorghum substrate is an important factor that can affect the growth of *Trichoderma* in SSF. The optimal moisture content for the growth of *Trichoderma* can vary depending on the specific *Trichoderma* species used, but generally, a moisture content of around 60-70% is suitable for *Trichoderma* growth on sorghum substrate. The addition of supplements to the sorghum substrate can help to promote the growth of *Trichoderma* in SSF. We are using 2% sucrose solution as additional supplements which enhance the growth of *Trichoderma viride.*

Conclusion

The use of fungi *Trichoderma* have been widely used in agriculture as bio-agents, which is the best way to replace chemical pesticides. It can reduce health, social, and environmental issues. Locally available substrates are potentially cost-effective for the production of *Trichoderma* spp. Among these, sorghum is the most effective substrate for the growth of *Trichoderma*. It produces effective molecules and secondary metabolites, which mediate *Trichoderma's* positive interaction with plants and provide resistance to pests. Bio-agent *Trichoderma* itself being non-pathogenic to plants, it is formulated in a way that favor's the activity and survival of microbes. It is economical to produce, has persistent storage ability, and is easy to handle.

Authors' Contribution

All authors contributed equally at all stages of research work and preaparation of the manuscript. Final form of manuscript was approved by all authors.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgement

We would like to express our gratitude to Terrapreta Agrovet for providing the resources necessary to carry out this research. We would also like to thank Dr. Deepa Khushalani-TIFR Mumbai for their guidance and support throughout the research process. Finally, we would like to acknowledge the contribution of Mansi Thakkar, Harsh Shukla and team who helped us in various ways during the research.

References

Agri U, Chaudhary P, Sharma A, & Kukreti B. (2022) Physiological response of maize plants and its rhizospheric microbiome under the influence of potential bioinoculants and nanochitosan. *Plant and Soil* **474**(1-2): 451-468. DOI: 10.1007/s11104-022-05351-2

Ajmal M, Ali H.I, Saeed R, Akhtar A, Tahir M, Mehboob MZ, & Ayub A. (2018) Biofertilizer as an alternative for chemical fertilizers. *Journal of Agriculture and Allied Sciences* **7**(1): 1-7.

Alwadai A.S, Perveen K, & Alwahaibi M (2022) The isolation and characterization of antagonist Trichoderma spp. from the soil of Abha, Saudi Arabia. *Molecules* **27**(8): 2525. DOI: 10.3390/molecules27082525

Aneja KR, Khan SA, & Aneja A (2016) Biopesticides an eco-friendly pest management approach in agriculture: status and prospects. *Kavaka*, **47**: 145-154.

Bhattacharjee R, & Dey U. (2014) Biofertilizer a way towards organic agriculture: A review. *African Journal of Microbiology Research* **8**(24): 2332-2343. DOI: 10.5897/AJMR2013.6374

Chaudhary A, Parveen H, Chaudhary P, Khatoon H, & Bhatt P. (2021) Rhizospheric microbes and their mechanism. *Microbial technology for sustainable environment* 79-93. DOI: 10.1007/978-981-16-3840-4_6

Chaudhary P, Singh S, Chaudhary A, Sharma A, & Kumar G (2022) Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Frontiers in Plant Science* **13**: 930340. DOI: 10.3389/fpls.2022.930340

Farnia A, & Hassanpour K (2015) Comparison between effect of chemical and biological fertilizers on yield and yield components of wheat (*Triticum aestivum L.*) Pishtaz cultivar. *Indian Journal of Natural Sciences* **5**: 7792-7808.

Glass AD, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker HJ, Kumar A, & Vidmar JJ (2002) the regulation of nitrate and ammonium transport systems in plants. *Journal of Experimental Botany* **53**(370): 855-864. DOI: 10.1093/jexbot/53.370.855

Grewal PS, Ehlers RU, & Shapiro-Ilan DI (Eds.) (2005) *Nematodes as biocontrol agents*. CABI. *DOI:* 10.1079/9780851990170.0000

Hartman G, Khadka R, & Uphoff N (2021) Benefits to plant health and productivity from enhancing plant microbial symbionts. *Frontiers in Plant Science* **11**: 1-21. DOI: 10.3389/fpls.2020.610065

Hewavitharana N, Kannangara SDP, & Senanayake SP (2018) Isolation, identification and mass production of five Trichoderma spp. on solid and liquid carrier media for commercialization. *International Journal of Applied Sciences and Biotechnology* **6**(4): 285-293. DOI: 10.3126/ijasbt.v6i4.22128

Howell C.R. (2003) Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. *Plant disease* **87**(1): 4-10. DOI: 10.1094/PDIS.2003.87.1.4

Kachhawa D (2017) Microorganisms as a biopesticides. *J Entomol Zool Stud* **5**(3): 468-473.

Kumar S, Kumar S, & Mohapatra T (2021) Interaction between macro-and micro-nutrients in plants. *Frontiers in Plant Science* **12**: 665583. DOI: 10.3389/fpls.2021.665583

Kumar S, Thakur M, & Rani A. (2014) Trichoderma: Mass production, formulation, quality control, delivery and its scope in commercialization in india for the management of plant diseases. *African journal of agricultural research* **9**(53): 3838-3852.

Kumhar KC, Babu A, Bordoloi M, & Ali A (2014) Evaluation of culture media for biomass production of *Trichoderma viride* (KBN 24) and their production economics. *American Journal of Agriculture and Forestry* **2**(6): 317-320. DOI: 10.11648/j.ajaf.20140206.24

Mukherjee PK, Mendoza-Mendoza A, Zeilinger S, & Horwitz BA (2022) Mycoparasitism as a mechanism of Trichoderma-mediated suppression of plant diseases. *Fungal Biology Reviews* **39**: 15-33. DOI: 10.1016/j.fbr.2021.11.004

Opender Koul OK (2012) Microbial biopesticides: opportunities and challenges. *CABI Reviews*, (2011), 1-26. DOI: 10.1079/PAVSNNR20116056

Parkash V, & Saikia AJ (2015) Production and multiplication of native compost fungal activator by using different substrates and its influence on growth and development of Capsicum chinensis Jacq. "Bhut Jolokia". *Biotechnology research international*, *2015*: 1-7. DOI: 10.1155/2015/481363

Ramanujam B, Prasad R.D, Sriram S, & Rangeswaran R (2010) Mass production, formulation, quality control and delivery of Trichoderma for plant disease management. *The journal of plant protection sciences* **2**(2): 1-8.

Ren X, Branà MT, Haidukowski M, Gallo A, Zhang Q, Logrieco AF & Altomare C (2022) Potential of Trichoderma spp. for biocontrol of aflatoxin-producing *Aspergillus flavus*. *Toxins* **14**(2): 86. DOI: 10.3390/toxins14020086

Seethapathy P, Kurusamy R, & Kuppusamy P (2017) Soil borne diseases of major pulses and their biological management. *Innovative Farming* ***2***(1): 01-11.

Sharma S, Kour D, Rana KL, Dhiman A, Thakur S, Thakur P, & Singh K (2019) Trichoderma: biodiversity, ecological significances, and industrial applications. *Recent advancement in white biotechnology through fungi: volume 1: diversity and enzymes perspectives* **1**: 85-120. DOI: 10.1007/978-3-030-10480-1_3

Sneha S, Anitha B, Sahair R.A, Raghu N, Gopenath TS, Chandrashekrappa GK, & Basalingappa MK (2018) Biofertilizer for crop production and soil fertility. *Academia Journal of Agricultural Research* **6**(8): 299-306.

Srivastava M, Kumar V, Shahid M, Sonika P, Singh A (2016) Trichoderma-a potential and effective bio fungicide and alternative source against notable phytopathogens: A review. *African Journal of Agricultural Research* **11**(5): 310-316. DOI: 10.5897/AJAR2015.9568

Vaishali K (2014) Biopesticides. *International journal of environmental research and development* **4**(2): 191-196.