



## Mini Review

# *Trichoderma* and Its Prospects in Agriculture of Nepal: An Overview

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### Abstract

In the world, the traditional agricultural practices are getting affected by various problems such as disease, pest, drought, decreased soil fertility due to use of hazardous chemical pesticides, pollution and global warming. As a result, there is a need for some eco-friendly bio-control agents that help in resolving the previous mentioned problems. The various types of biological control agents such as bacteria and fungi are involved in bio-control activity. Among them, fungal genus *Trichoderma* plays a major role in controlling the plant diseases. Species of *Trichoderma* are diverse fungal microbial community known and explored worldwide for their versatilities as biocontrol and growth promoting agents. These fungi reproduce asexually by production of conidia and chlamydospores and in wild habitats by ascospores. *Trichoderma* species are efficient mycoparasites and prolific producers of secondary metabolites, some of which have clinical importance. However, the ecological or biological significance of this metabolite diversity is sorely lagging behind the chemical significance. Several *Trichoderma* spp. positively affect plants by stimulating plant growth, and protecting plants from fungal and bacterial pathogens. They are used in biological plant protection as bio-fungicides as well as in bioremediation. A large number of research groups are working on various aspects of *Trichoderma* viz., diversity, ecology and their applications. The capacity of *Trichoderma* fungi to produce lytic enzymes is used in animal feed, and wine making and brewery industries. *Trichoderma* spp. are the most successful bio-control agents as more than 60% of the registered bio-fungicides used in today's agriculture belongs to *Trichoderma* -based formulation. The increase in incidence and severity of diseases and emergence of new diseases causes the significant yield losses of different crops in Nepal. But the research and studies on plant diseases are limited.

**Keywords:** Bio-control; Crop plants; pathogens; *Trichoderma* spp.

### Introduction

*Trichoderma* is a genus of fungi in the family hypocreaceae that is present in all soils, where they are the most prevalent culturable fungi. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. This refers to the ability of several *Trichoderma* species to form mutualistic endophytic relationship with several plant species. The genomes of several *Trichoderma* species have been sequenced and are publicly available. Nepal is an Agricultural country where about 65% of total people are involved in agriculture. Most of them are dependent in the

agriculture for sustaining the life. At present context there are various constraints in Nepalese agriculture. Among them plant diseases one of the major constraints in crop production in Nepal. Plant diseases cause both qualitative as well as quantitative losses in Agriculture with negative impact on the economy of Nepal. Past and the present plant disease scenario indicated that the occurrence of several viruses, fungus, bacteria on cereals, cucurbits, legumes and solanaceous vegetables and other crops in epidemic form in the country. The incidence and severity of disease is

increasing. New diseases are also emerging. Research and studies on plant diseases are limited in Nepal. In recent years, diseases caused significant yield losses. Disease has created a threat for production and productivity of important crop plants. Deteriorate both quality and quantity of produce and ultimately reduces market price and affect the national economy of the country. Many farmers in Nepal are not aware of pesticide level of poisoning, safety precautions and potential hazards on health and

environment and agriculture field. According to 2012 survey the estimate, the yearly import of pesticides in Nepal is about 211ta.i. with 29.19% insecticides, 61.38% fungicides, 7.43% herbicides and 2% others (Fig. 1). The gross sale value accounts US\$ 3.05 million per annual. Injudicious and indiscriminate use of pesticides and presence of pesticide residues in food, fruits, vegetables and environment is a matter of grave concerns.

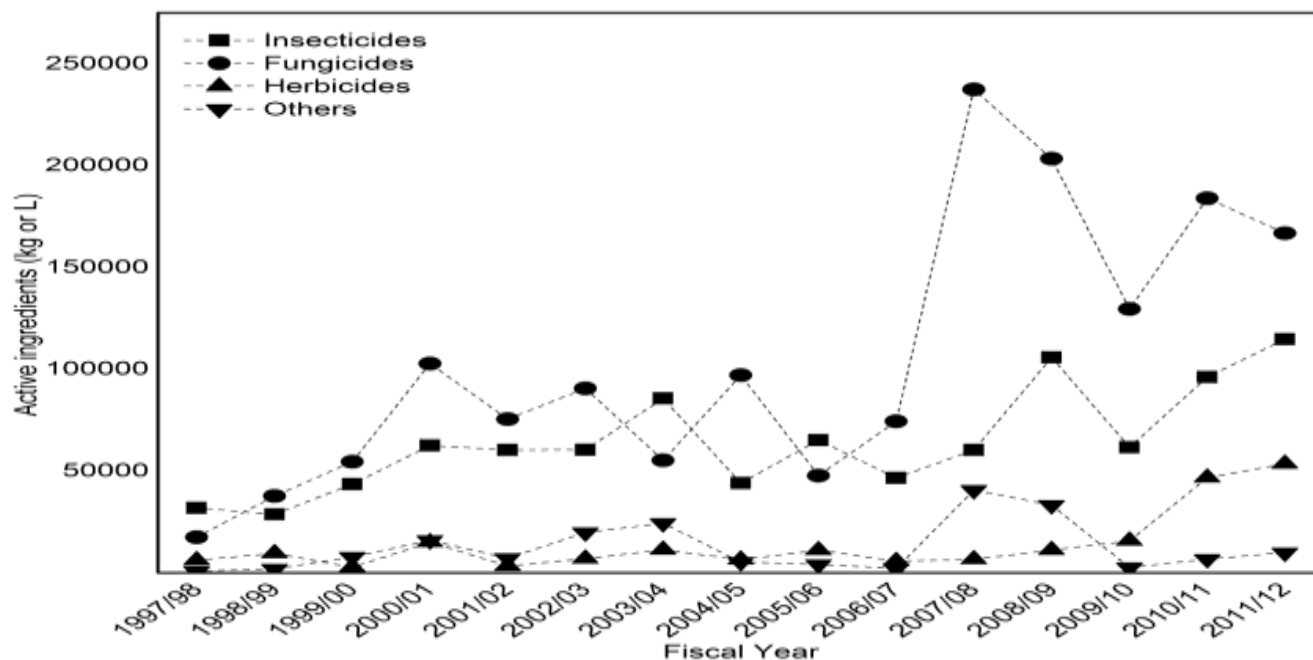


Fig. 1: Trends of insecticides, fungicides and herbicides consumption in Nepal (Sushma, et al., 2015)

Table 1: Management of plant pathogens/diseases by different *Trichoderma* species in major crops

SN	Crop	Pathogen/disease	<i>Trichoderma spp</i>	Types of treatment	References
1	Bambo ( <i>Dendrocalamus Calostachyus</i> )	<i>Polyporus Sanguineus</i>	<i>Trichoderma lingorum</i> <i>T. harzianum</i> <i>T. viride</i>	Soil	Kundu and chaterjee, 2003
2	Black gram ( <i>Vigna mungo</i> )	<i>Macrophomina phaseolina</i> <i>F. oxysporium</i> <i>Alternaria alternata(foliar)</i>	<i>T. harzianum</i> <i>T. viride</i>	Seed pelleting	Raghuchander et al., 1997
3	Brinjal ( <i>Solanum melongena L.</i> )	<i>Fusarium solani</i> <i>F. oxysporium .f. sp</i>	<i>T.harzianum</i> <i>T.viride</i>	Seed and soil	Balaji and Ahir, 2011
4	Cabbage ( <i>Brassica oleracea L. var capitata</i> )	<i>S.sclerotiorum</i> <i>Rhizoctonia solani</i>	<i>T.harzianum</i> <i>T.viride</i>	Soil and seed	Sharma et al., 2001 Sharma et al., 2003
5	Capsicum( <i>Capsicum annum .L</i> )	<i>Alternaria alternate</i>	<i>T.harzianum</i> <i>T.viride</i>	Seed	Kapoor, 2008
6	<i>Cardamomum elettaria</i>	<i>Pythium vexans</i> <i>R.solani</i>	<i>T.harzianum</i> <i>T.viride</i>	Bed soil	Bhai and Thomas, 2010
7	<i>Carnation Dianthus</i>	<i>F.oxysporium f.sp. dianthi</i>	<i>T. harzianum</i>	Seed	Kapoor, 2008
8	Cauliflower ( <i>Brassica oleraceae L.var .botrytis</i> )	<i>R. solani</i>	<i>T. harzianum</i> <i>T. viride</i>	Fungicide and soil	Sharma et al, 2001 Sharma et al, 2003

**Table 1:** Management of plant pathogens/diseases by different *Trichoderma* species in major crops

SN	Crop	Pathogen/disease	<i>Trichoderma</i> spp	Types of treatment	References
9	Chickpea ( <i>Cicer arietum</i> L)	<i>F. oxysporium f.sp ciceris</i> <i>R. solani</i> <i>S. rolfsii</i> <i>Macrophomina phaseolina</i> <i>Botrytis cinerea</i>	<i>T.harzianum</i> <i>T.viride</i> <i>Trichoderrma ressei</i>	Soil and seed	Mukherjee et al, 1997 Pandey, 2003
10	Chilli ( <i>Capsicum annum</i> L)	<i>F.oxysporium</i> <i>Pythium spp</i> <i>R.solani</i>	<i>T. harzianum</i> <i>T. viride</i>	Soil and seed	Rini and Sulochana, 2006 Kapoor , 2008
11	Citrus	<i>F. solani</i>	<i>T. harzianum</i> <i>T. viride</i>	Foliar	Kalita et.al., 1996 Singh et.al., 2000
12	Coconut ( <i>cocos nucifera</i> L)	<i>Ganoderma lucidum</i>	<i>T. harzianum</i> <i>T. viride</i> <i>Trichoderrma ressei</i>	Foliar	Karthikeyan et al., 2006
13	Coffee ( <i>Coffea arabica</i> L)	<i>Phomopsis thaeae</i> <i>Glomerella cingulate</i>	<i>T. harzianum</i>	Seed	Deb and Dutt , 1999
14	Cotton ( <i>Gossypium hirsutum</i> )	<i>R. solani</i> <i>S. rolfsii</i> <i>P. aphanidermatum</i>	<i>T. harzianum</i> <i>T. viride</i>	Seed and soil	Sreenivasaprasad, 1990 Gaur et al., 2005
15	Cowpea ( <i>vigna sinensis</i> )	<i>R.solani</i>	<i>T.harzianum</i>	Seed	Pan and Das, 2011
16	Fenugreek ( <i>Trigonella foenum graecum</i> L)	<i>P. aphanidermatum</i> <i>S. sclerotiorum</i>	<i>T.harzianum</i> <i>T.viride</i>	Seed and soil	Trivedi et al., 2010
17	Ginger ( <i>Zingiber officinale</i> )	<i>P. aphanidermatum</i>	<i>T.harzianum</i>	Seed ,soil and foliar	Gupta et al., 2010
18	Grapes ( <i>vitis vinifera</i> )	<i>Penicillum expansum</i> <i>Fusarium moniliforme</i> <i>B.subtillis</i>	<i>T.harzianum</i> <i>T.viride</i>	Seed and soil	Senthil et al., 2011
19	Groundnut ( <i>Arachis hypogaea</i> L)	<i>S. rolfsii</i> Sacc., <i>A. niger</i> <i>R. solani</i>	<i>T. longibrachiatum</i> <i>T.harzianum</i> <i>T.viride</i>	Seed and soil	Sreedevi et al., 2012 Biswas and Sen, 2000
20	Guava ( <i>Psidium guajava</i> L)	<i>F. oxysporum f.sp. Psidii</i> <i>Macrophomina</i> <i>Phytophthora</i>	<i>T.harzianum</i> <i>T.viride</i>	Soil	Pandey and Dwivedi, 1987 Misra, 2007
21	Jute ( <i>Corchorus olitorius</i> )	<i>Macrophomina</i> <i>Alternaria alternate</i>	<i>T.harzianum</i> <i>T.viride</i>	Soil and foliar	Bhandari et al., 2013
22	Maize ( <i>Zea mays</i> )	<i>P. notatum</i> <i>R. solani</i> <i>Alternaria alternata</i>	<i>T.harzianum</i> <i>T.viride</i>	Seed and foliar	Bhandari et al., 2013
23	Mango ( <i>Mangifera indica</i> L)	<i>Pestalotia sp.</i> <i>A. flavus</i> <i>C. gloeosporioides</i>	<i>T. reesei</i> <i>T.harzianum</i> <i>T.viride</i>	Seed and foliar	Bhuvaneshwari and Rao, 2001
24.	Mushroom	<i>Rhizopus stolonifer</i>	<i>T. viride</i>	Foliar	Rawal et al., 2013
25	Mustard ( <i>Brassica juncea</i> L)	<i>Azospirillum</i> <i>P. palmivora</i>	<i>T. viride</i> <i>T.harzianum</i>	Seed	Patni et al., 2005 Meena et al., 2004 Meena et al., 2011
26	Onion ( <i>Allium cepa</i> )	<i>A.alternate</i> <i>S. vesicarium</i> <i>Cladosporium allii-cepae</i>	<i>T. viride</i> <i>T. reesei</i> <i>T.harzianum</i>	Soil, seed, Seedling dip	Mishra and Gupta, 2012 Prakasam and Sharma, 2012
27	Rice ( <i>Oryza sativa</i> )	<i>R. solani</i> <i>Fusarium spp</i>	<i>T. viride</i> <i>T. harzianum</i>	Seed	Bhat et al., 2009 Chakravarthy et al., 2011

## **Trichoderma as Bio-control Agent for Different Crop Diseases**

### **Cereal Crops**

#### **Rice (*Oryza sativa* L)**

Rice dominates the country's crop sector accounting for over 42.5 percent (168,047 ha) of the total area under food grains and shares 51.6 percent in total food grain production (MOAD, 2017). As the most important staple food of Nepalese people, rice supplies about 40% of the food calorie intake and contributes nearly 20% to the agricultural gross domestic product (AGDP) and almost 7% to GDP (DoA/CDD, 2015). Rice only accounts for more than 50% of the total calories of Nepalese people (Kharel et al., 2018). But it's production is constrained by diseases of fungal, bacterial and viral origins. Species of *Trichoderma*, *T. harzianum*, *T. viride*, *T. reesei*, *T. longibrachitum* and *T. koningii* in combination with chemical control was evaluated by many researchers finding them effective against these diseases. Sheath blight of rice incited by *Rhizoctonia solani* is one of the common and destructive diseases of rice. There are several important reports on the biological management of this disease. A study reported that the rice seed treated with two antagonistic fungi, viz., *T. viride* tode (Harz) and *T. harzianum* rifai along with chemicals were found effective to control sheath blight of rice and increase the yield of the crop (Das and Hazarika, 2000). The use of *Trichoderma* inoculation as a seedling treatment can help to enhance rice production and productivity in Nepal, especially in conjunction with SRI methods and even more so with organic fertilization, which in this integrated system of rice cultivation can reduce the use and cost of chemical fertilizer. In such a system under the prevailing soil and climatic conditions, an indigenous heirloom rice variety was able to outperform in both agronomic and economic terms the more modern improved variety. (Khadka and Uphoff, 2019)

#### **Maize (*Zea mays* L.)**

Maize is second most important crop after rice in terms of area and production in Nepal. Maize shares 24.93% in total edible cereal grain production. At present, It is cropped in an area of 891,583ha with production of 2,231,517Mt (MOAD,2017). In the context of Nepal, the importance of maize increase with altitude, eventually standing as a staple crop in northern part of the country (KC et al.,2015). It is another important cereal crop which suffers from soil borne and foliar pathogens. Some attempts have been made on the use of *Trichoderma* against important diseases of maize. Investigations made by (Sankar and Sharma, 2001) clearly indicated the performance of *T. viride* in in vitro and in vivo conditions which suppressed the charcoal rot disease caused by *Macrophomia phaseolina* and also enhanced the growth of crop. After a decade, (Khedekar et al., 2010) evaluated

the efficacy of *T. harzianum* against leaf blight caused by *Helminthosporium turcicum* and found similar results.

#### **Wheat (*Triticum* spp. L.)**

Wheat is the third most important crop after rice and maize in Nepal; it's cultivated in 762 373 hectares with an average national yield of 2.55 t/ha (MoAD, 2017) and is an important source of carbohydrates, proteins, vitamins and minerals. Among the biotic constraints, smut diseases cause significant loss of yield, and quality of the grains. First report of use of bio-control of seed borne disease, i.e. loose smut of wheat was shown by (Aggarwal et al. 1991), who used *T. viride* as seed treatment. *Trichoderma* species viz ., *T. viride* (TV5), *T. koningii*, *T. hamatum*, *T. harzianum* and *T. lignorum* were found to inhibit germination of chlamydospores of wheat pathogen *U. segetum*. var. *tritici* (Mondal et al., 1995). When *Trichoderma* and NPK are accompanied with farmyard manure, most of the growth and yield parameter shows the highest value in wheat. (Mahato et al., 2018)

### **Pulse Crops**

#### **Chickpea (*Cicer arietinum* L.)**

Chickpea is one of the most popular pulses in many regions of the Nepal. It is cropped in an area of 9,883ha with production of 10,914mt (MOAD, 2017).It contributes 3% of the major pulse production in Nepal. Chickpea is frequently attacked mainly by a wilt caused by *Fusarium oxysporum* f. sp. *cicero*, stem rot by *Sclerotinia sclerotiorum* and damping off caused by *R. solani* which are worldwide in distribution. Preliminary investigations based on usefulness of *Trichoderma* as biocontrol agent was done by Kaur and Mukhopadhyay (1992) who reported successful integration of *T. harzianum* with fungicides like Vitavax 200 and Ziram in inhibiting chickpea wilt. Coating chickpea seeds with biocontrol agent *Bacillus subtilis*, *Gliocladium virens*, *Trichoderma harzianum* and *T. viride* significantly controlled *Fusarium oxysporum* f.sp. *cicero* wilt by 30 - 45.8 per cent and integration of bio-control agent and carboxin increased the seed yield by 25.4 - 42.6 per cent (De et al., 1996).

### **Oilseed crops**

#### **Mustard *Brassica juncea* (L.)**

*Mustard Brassica juncea* (L.) is one of the major oilseed crops cultivated in Nepal. It is cropped in an area of 169,769 ha with production of 171,499 Mt (MOAD, 2017). Several researches were focused on the eco-friendly approaches of managing these major diseases employing *Trichoderma*. In an in vitro study *T. viride* performed along with fungicides mancozeb and carbendazim and showed superior result against mycelial growth of *Alternaria brassicae* over control (Meena et al., 2004). *Trichoderma* spp. significantly increased plant height (<0.001), leaf number (0.001), root length (0.007), root fresh weight (<0.001) and root dry

weight (<0.001) compared to the untreated plants (control) in mustard. (Roslee et al., 2017).

#### **Groundnut (*Arachis hypogaea* L.)**

Groundnut (*Arachis hypogaea* L.) yield less due to the high disease incidence. It is cropped in an area of 3,269ha with production of 3,843 Mt(MOAD,2017). Though more than 55 pathogens have been reported to affect the crop, few diseases like early leaf spot (*Phaeoisriopsis arichidicola*), late leaf spot (*Phaeoisriopsis personata*), rust (*Puccinia arichidis*), collar rot (*A. niger van tiegham*), stem rot (*S. rolfisii*), root rot (*M. phaseolina*) and alfalfa root (*Aspergillus flavus*) are recognized to be economically important and have been biologically managed by deploying *Trichoderma* spp. Asghari and Mayee (1991) reported the application of *T. harzianum* and 0.2% carbendazim effectively protected groundnut from damage by *Sclerotium rolfisii*. Treatment with *T. viride* and *P. fluorescens* increased pod yield by 14-35% and 22-33% over control groundnut root rot caused by *Macrophomina phaseolina* in groundnut. (Ramesh & Korikanthimath, 2006)

#### **Sesame (*Sesamum indicum*)**

Sesame (*Sesamum indicum*) is one of the most ancient oil-seed crops. It is cropped in an area of 4,328 ha with production of 2,751 mt (MOAD, 2017). Tamimi and Hadvan (1985) reported that the differences in the amount of inhibition of growth of a range of *sesamum* wilt causing fungi by gaseous metabolites from *N. sitophila* and from *T. harzianum* could be accounted by differences in their ages. The highest level of growth inhibition from test fungi ever recorded was as follows: 3-day-old *N. sitophila* was 55% on virulent *R. solani*, 51% on a virulent *R. solani*, 48% on *F. oxysporum*, and 40% on *M. phaseoli*.

### **Cash crops**

#### **Sugarcane**

Sugarcane is a major commercial crop grown in the tropical and sub-tropical regions of the country. It is cropped in an area of 639 ha with production of 4,346,754 mt(MOAD, 2017). Among the fungal diseases, red rot and smut cause severe yield loss. (Mishra, 1982) observed that the *Rhizosphere microflora* of sugarcane plants was affected by incidences of red rot. Later, Joshi and Misra (2013) reported that the secondary metabolites of *Trichoderma* isolates native to sugarcane ecosystem inhibited *Colletotrichum falcatum* causing red rot of sugarcane. Both in vitro and in vivo experiments revealed that *T. harzianum* (T20) is highly efficient, has mycoparasitic activity against *C. falcatum*, proved its endophytic nature, capable of inhibiting the mycelial growth of *C. falcatum* and reducing red rot disease in sugarcane during their interaction. (Elamathi et al., 2017)

#### **Cotton**

Cotton is another widely cultivated commercial crop of Nepal with great economic importance. It is cropped in an area of 125ha with production of 129 mt(MOAD, 2017).

Major diseases associated with decreased yield of cotton crop are root rot (*M. phaseolina*), wilt (*Fusarium vasinfectum*), root rot (*Rhizoctonia bataticola*), anthracnose (*C. colletotricum gossypium*), *Alternaria* leaf spot, bacterial blight and damping off (*Pythium ultimum*). Soil application of *Trichoderma* was observed to be significantly superior over seed treatment (Gaur et al., 2005).

#### **Tea and coffee**

Tea and coffee are the popular beverage crops cultivated in more than 50 countries. Tea is cropped in an area of 20,747ha with production of 23,821 mt(MOAD, 2017). Tea sub-sector contributes about 0.0105% in the national GDP and 0.0347% in the Agricultural Gross Domestic Product (AGDP) (CBS, 2014). At present, Coffee is cropped in area of 2,618 ha with production of 532 mt(MOAD, 2017). Among the diseases, collar canker caused by *Phomopsis theae* and brown blight caused by *Glomerella cingulata*, collar rot of coffee caused by *R. solani* are significant. Phyllosphere and soil mycoflora of an experimental tea plantation of Cachar was documented (Deb et al., 1999). (Bandopadhyay et al. 2008) observed that *Gliocladium* JGP1 and *Trichoderma* JPT9 isolates significantly inhibited the sclerotial growth of *M. phaseolina* at different stages.

### **Vegetable crops**

#### **Onion (*Allium cepa*)**

Onion (*Allium cepa*) is another important vegetable crop of Nepal. Six major diseases *Alternaria alternata* causing foliar blight of onion, *Alternaria porri* causing purple blotch of onion, damping-off and basal rot by *F.o. f.sp. cepae* and *Pythium* sp, white rot by *S. rolfisii* and black mould by *A. niger* were found to be associated with the crop. Control of *A. porri* causing purple blotch of onion has been widely studied. Effective isolates of *T. harzianum* i.e. Th3, Th-30 of *T. harzianum* and Tv-12, Tv-15 of *T. viride* were reported to express high level of disease reduction and growth promotion in susceptible onion (Prakasam and Sharma, 2012).

#### **Cauliflower and Cabbage**

Cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*Brassica oleracea* L. var. *capitata*) are the most important winter vegetables of Nepal. Cauliflower is cropped in an area of 34967.0 ha with production of 550044.8 mt (MOAD, 2017). Cabbage is cropped in an area of 28071.4 ha with production of 484036.8 mt (MOAD, 2017). Major pathogens which cause crop losses are club root caused by *Plasmodiophora brassicae*, black rot by *Xanthomonas campestris*, wire stem by *Rhizoctonia solani*, leaf spot by *A. brassicae*, leaf blight by *A. brassiciola*, damping off by *R. solani*, *Pythium*, *Phytophthora*, *Corticium* and *Fusarium*. Antagonistic potential of *Trichoderma* and *Aspergillus* species was evaluated on *Sclerotinia sclerotiorum* causing rots in cabbage and

cauliflower by (Sharma et al. 2001) and further the role of *Trichoderma spp.* in an IDM programme on the management was confirmed against *Sclerotinia sclerotiorum* (Sharma et al., 2001; Zewain et al., 2004).

## Fruit crops

### *Guava (Psidium guajava)*

Guava (*Psidium guajava*) is an important fruit crop of Nepal. It is cropped in an area of 3,453ha with production of 22,370 mt(MOAD, 2017). Pre-harvest diseases like canker, die back, decline etc. affect the plant growth and production, while postharvest diseases such as *Phytophthora*, *Macrophomina* and several others spoil the fruits in field, storage and in transit. *Gliocladium roseum* is also identified as a most potent pathogen besides *F.o.f.sp. psidii* and *F. solani*. S and Dwivedi (1987) observed the Mycoflora associated with seeds from healthy and rotten fruits of guava. Later, with the advent of bio-control technology for plant disease management, several groups attempted to evaluate and use *Trichoderma* technology in combating diseases of fruit like guava.

### *Mango (Mangifera indica)*

Mango (*Mangifera indica*) occupies a prominent place amongst fruit crops in Nepal. It is cropped in an area of 52,815 ha with production of 266,628mt(MOAD, 2017). It suffers from great economic loss due to many pathogens e.g. *F. moniliforme var. subglutinans* causing mango malformation. Biocontrol was used by some groups against different pathogens and found successful by many of them. (Bhuvaneshwari and Rao, 2001) studied the in vitro interaction of *T. viride* with the postharvest pathogens of mango and found that the in vitro interaction of *T. viride* with the postharvest pathogens inhibited the growth of *Pestalotia sp.*, *A. flavus*, *L. theobromae*, *C. gloeosporioides*, *R. stolonifer*, *A. niger* and *M. phaseolina* by 72.88, 70.74, 62.41, 56.83, 54.60, 52.77 and 51.08%, respectively.

## Conclusion and Recommendations

Agriculture is the major sector of Nepalese economy. It provides employment opportunities to 66 percent of the total population and contributes about 33 percent in the GDP. So it is prime duty to promote biological method in controlling disease of plant. The success of biological control of plant diseases depend on the availability of effective formulations of biocontrol agents, their survival during storage and rapid multiplication and colonization after inoculation. Different species of *Trichoderma* are known to produce different kinds of enzymes which have a significant role in biocontrol activity like cell wall degradation, biotic and abiotic stress tolerance, hyphal growth, antagonistic activity against plant pathogens. By the advance techniques laid in the molecular biology, we can easily isolate, characterize, clone, sequence and express the functions of these genes and can study their functions and role in the biocontrol mechanism (Sharma et al., 2011).

Strains of *Trichoderma* species against a wide range of challenging pathogens, their mechanism of action, survivability and rhizospheric competence with the changing climate, compatibility with the various components of integrated pest management, quality formulations and field success are the major issues which need to be further explored intensively. Injudicious and indiscriminate use of pesticides and presence of pesticide residues in food, fruits, vegetables and environment is a matter of grave-concerns.so it would be better in use of biological method in controlling disease of plant.

## Author's Contribution

Nabin Pandey & Madhusudhan Adhikhari designed the research plan; Binod Bhandana performed experimental works & collected the required data. Madhusudhan Adhikhari & Binod Bhandana analysed the data; Nabin Pandey, Madhusudhan Adhikhari & Binod Bhandana prepared the manuscript, critical revised and finalized the manuscript.

## Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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