Studies on Effect of Carbon and Nitrogen Sources for fermentation of Soil Fungi *Trichoderma reeseii*

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Abstract

In this research work, soil samples were collected from 6 different places of Swedaw Pagoda, Amarapura Township, Mandalay Region. Fourteen different fungi were isolated by using chemical treatment dilution methods and soil dilution methods. All isolated fungi were tested by six kinds of test organisms by paper disc diffusion methods. In the screening program, fungus *Trichoderma reeseii* was exhibited the antibacterial activity on *Escherichia coli.* Therefore this fungus *Trichoderma reeseii* was selected for further investigation. In this study, carbon and nitrogen utilization were investigated, soluble starch, molasses and potato powder were excellent for carbon sources and NZ amine type A, meat extract, KNO_3, peanut and fish cake were excellent for nitrogen sources. In the fermentation studies 84 hrs seed culture and 20% sizes of inoculum were optimized for the fermentation. FM–1 was excellent for the production of antibacterial metabolite. The choice of a good fermentation medium is virtually important to success of an industrial fermentation.

Keywords: Carbon and Nitrogen activity on fermentation of *Trichoderma reeseii*

Introduction

Microorganisms live in every part of the biosphere including, soil, hot springs, on the ocean floor, high in the atmosphere and deep inside rocks within the Earth's crust. They may be ancestors of all living things and the support system for all other forms of life. Socialites have utilized soil fungi for centuries in a wide variety of ways by capitalizing on the metabolism and metabolites produced. Some microbes are used for medicinal production. Antibiotics, one of the most important groups of medicine, are produced by fungi and bacteria.

Microorganisms require as a suitable environment include a growth medium that can support their nutritional needs. Fermentation is a chemical change or decomposition of substances by the action of bacteria, yeast, fungi and other microorganisms. Nutrient media for production also have to be optimized not only the ingredients used but also in how the nutrient medium is prepared.

General media requirements included carbohydrates, which were traditional carbon and energy sources for microbial fermentation. Secondary metabolites were small molecules that were not directly involved in metabolism and growth of the organism (Domain, 1999).

For economic reasons, pure glucose or sucrose can seldom be used as the carbon source. Starch can be directly metabolized as carbon source by amlylase– production

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organisms. Yeast extracts are excellent substances for many microorganisms. As nitrogen source, most microbes require protein, peptide or amino acids but many can use ammonia, nitrates or nitrogen molecules (Crueger and Crueger, 1989).

Growth media and incubation conditions have a very strong influence of secondary metabolite production. The large scale production of an antibiotic depends on a fermentation process. During fermentation, the organisms produce the antibiotic material, which can then be isolated for use as a drug (Fenical, 1993). Optimal fermentation conditions are very important for maximal productivity of metabolites.

The present study was undertaken to find out the effect of different culture media, inoculum size and age along with various carbon and nitrogen sources on the growth and bioactive metabolite production.

Materials and Methods

The Effects of Carbon and Nitrogen Sources for the Growth Fungus *Trichoderma reeseii*

In the studies of antibacterial activity, fungus *Trichoderma reeseii* showed highly selective activity against *Escherichia coli*. Based on this result, carbon and nitrogen utilization for the growth of selective isolated fungus *Trichoderma reeseii*, was investigated.

The Ages of Culture and Sizes of Inoculums for the Fermentation of fungus *Trichoderma reeseii*

For the suitable fermentation conditions, ages of culture (66 hrs, 72hrs, 78hrs, 84hrs, 90hrs and 96 hrs) and the sizes of inoculums 5%, 10%, 15%, 20%, 25% and 30% seed culture were also investigated.

The Effects of Carbon and Nitrogen Sources for the Fermentation of Fungus *Trichoderma reeseii*

It is needed the balances of carbon and nitrogen sources for the production of metabolite. Therefore, the effect of carbon and nitrogen sources for fermentation by *Trichoderma* was studied. In the study of the effects of carbon sources, 1 g of each carbon source such as glucose, sucrose, glycerol, soluble starch, fructose, molasses, tapioca powder and potato powder were utilized. In the composition, basal medium was Yeast extract 0.2 g, NZ amine type A 0.3g, K_2HPO_4 0.001g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.02 g, DW 100 mL, adjusted at pH 6.5.

In the study of the effects of nitrogen sources, 0.7 g of each of Yeast extract and NZ–amine type A, Polypeptone, Rice bran, Peanut, Meat extract, KNO_3 and Fish cake were used as nitrogen sources. In the composition of basal medium was Galactose 1.5 g, Glycerol 1.0g, K_2HPO_4 0.001g, $MgSO_4$ 0.001g, $CaCO_3$ 0.02g, DW 100mL, pH 6.5.

Production of Antibacterial Metabolite from Four Different Fermented Media by Fungus *Trichoderma reeseii*

The balances of Metabolite nutrients for the fermentation is necessary to be produced the metabolite. Therefore, fermentation was undertaken with suitable conditions of 20% sizes and 84 hrs ages of inoculum with four different media.

Fermentation medium (NITE, 2005)

FM–1	
Glucose	1.0 g
Soluble Starch	1.0 g
Polypeptone	0.9 g
K_2HPO_4	0.001g
DW	100 ml
pH	6.5

FM–2	
Glucose	2.0g
Yeast extract	0.5 g
Glycerol	1.0 ml
K_2HPO_4	0.001 g
DW	100 ml
pH	6.5

FM–3	
Glycerol	2.0 ml
Yeast extract	1.0 g
Polypeptone	0.8 g
K_2HPO_4	0.001g
DW	100 ml
pH	6.5

FM–4	
Glycerol	2.0 ml
Glucose	1.0g
Yeast extract	1.0 g
Polypeptone	0.8 g
K_2HPO_4	0.001g
DW	100 ml
pH	6.5

Results

The Effect of Carbon and Nitrogen Sources for the Growth of Fungus *Trichoderma reeseii* (Omura, 1985; Crueger and Crueger, 1989; Phay, 1997)

In this study, eight different carbon sources such as glucose, sucrose, glycerol, tapioca powder, molasses, soluble starch, fructose and potato powder were used. The sources from soluble starch, molasses and potato powder showed the excellent growth. The sources from glucose, glycerol and tapioca powder showed the good growth. The sources from sucrose and fructose showed poor result (Table 1, Figure 1). Eight kinds of nitrogen sources were also used to observe the growth of selected fungus. The sources from NZ–amine type A, meat extract, KNO_3, peanut and fish cake showed the excellent growth. The sources from yeast extract, polypeptone were showed the good growth and rice bran showed poor result (Table 2, Figure 2).

Table 1 Morphological character of Fungus *Trichoderma reeseii* on various Carbon Sources

Carbon sources	Growth

glucose	Good
Sucrose	Poor
glycerol	Good
Soluble starch	Excellent
Fructose	Poor
Molasses	Excellent
Potato powder	Excellent
Tapioca powder	Good

Notes; Growth result (Samson and Pitt, 2000 and Ando and Inaba, 2004)

1.0mm to 3.0mm = Poor

3.0mm to 5.0mm = Moderate

5.0mm to 8.0mm = Good

Above 8.0mm = Excellent

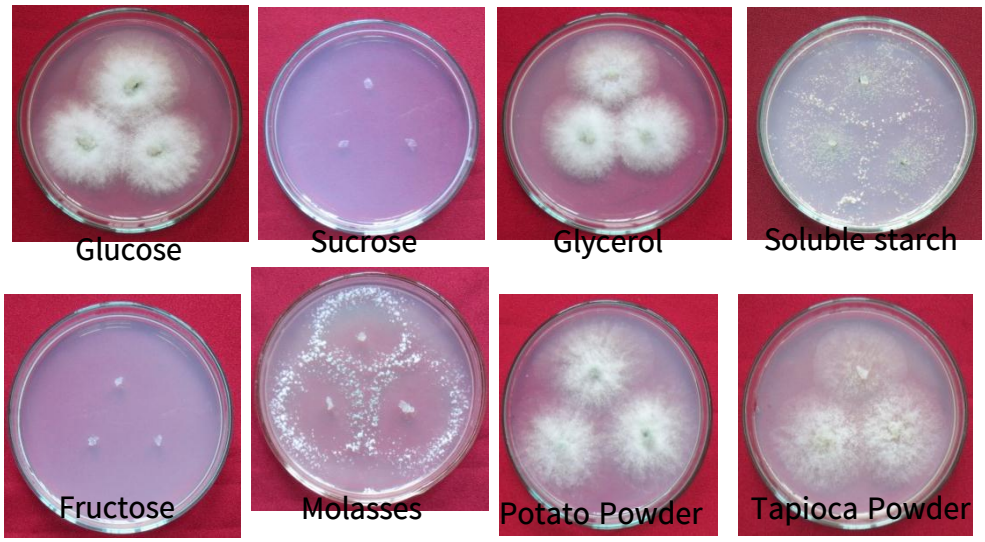

Figure 1. Growth of fungus *Trichoderma reeseii* on various carbon sources (7 days old culture)

Table 2. Morphological character of fungus *Trichoderma reeseii* on various Nitrogen Sources

Nitrogen sources	Growth

Yeast extract	Good
NZ amine type A	Excellent
Polypeptone	Good
Meat extract	Excellent
KNO_3	Excellent
Rice Bran	Poor
Peanut	Excellent
Fish cake	Excellent

Notes; Growth result (Samson and Pitt, 2000 and Ando and Inaba, 2004)

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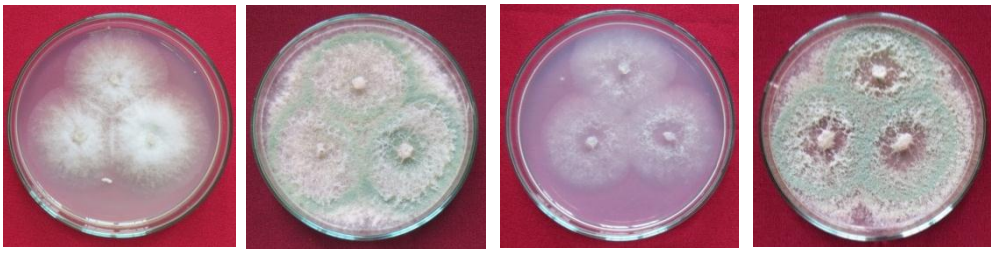
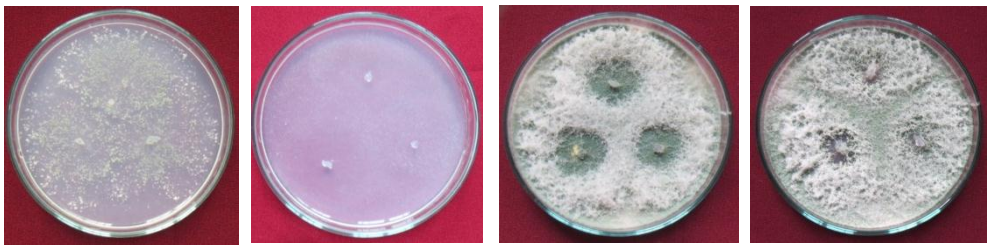


Yeast extract NZ amine type A Polypeptone Meat extract

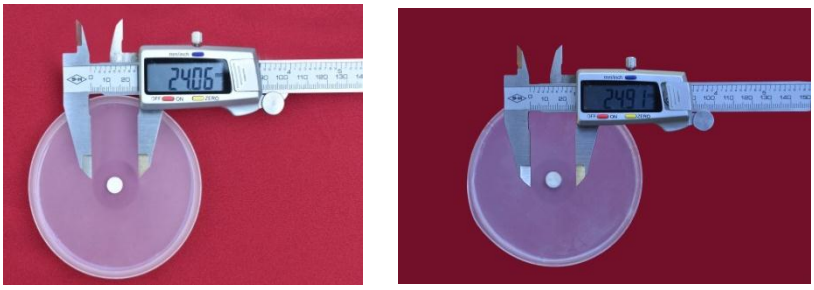


KNO_3 Rice Bran Peanut Fish cake

Figure 2. Growth of fungus *Trichoderma reeseii* on various nitrogen sources (7 days old culture)

The Ages of Culture and Sizes of Inoculums for the Fermentation of fungus *Trichoderma reeseii*

In the fermentation studies, the highest activity reached at 84 hrs ages of culture and 20% sizes of inoculum for the fermentation. Depending on the studies, 84 hrs of ages and 20% inoculums was proper condition to carry out the fermentation (Figure –3).



84 hrs 20%

Figure 3. The Effects of Ages and Sizes of Inoculum on Fermentation

The Effect of Carbon and Nitrogen Sources for Fermentation of *Trichoderma reeseii* (Omura, 1985, Crueger and Crueger, 1989; Phay, 1997)

The effect of carbon and nitrogen sources on fermentation were showed in (Table 3) and (Table 4) respectively. In the investigation of carbon sources molasses was excellent (23.73 mm) and sucrose was poor (17.60 mm) (Figure 4). In the investigation of nitrogen sources on the fermentation, meat extract was excellent (27.69 mm) and rice bran was poor result (Figure 5).

Table 3. The effect of carbon sources on fermentation of fungus *Trichoderma reeseii*

Carbon sources	Activity (Clear zone, mm)
Glucose	19.32
Sucrose	17.60
Glycerol	20.81
Soluble starch	21.82
Fructose	18.44
Molasses	**23.73**
Potato powder	20.97
Tapioca powder	19.70

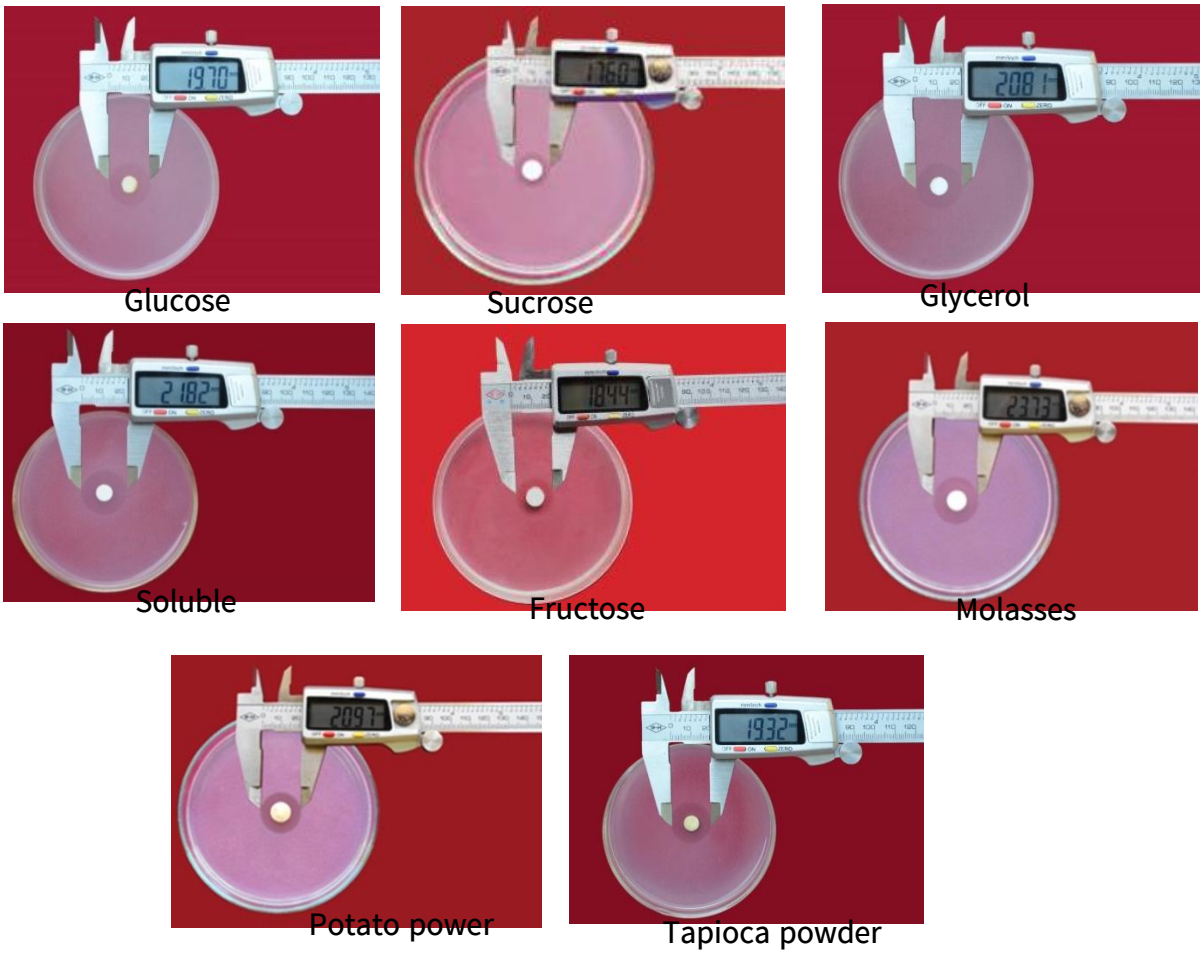


Figure 4. The effect of carbon sources on fermentation of isolated fungus *Trichoderma reeseii*

Table 4. The effect of nitrogen sources on fermentation of *Trichoderma reeseii*

Nitrogen sources	Activity (Clear zone, mm)
Yeast extract	19.27
NZ–amine type A	20.28
Polypeptone	22.42
Meat extract	**27.69**
KNO_3	21.45
Rice Bran	–
Peanut	18.95
Fish cake	18.46

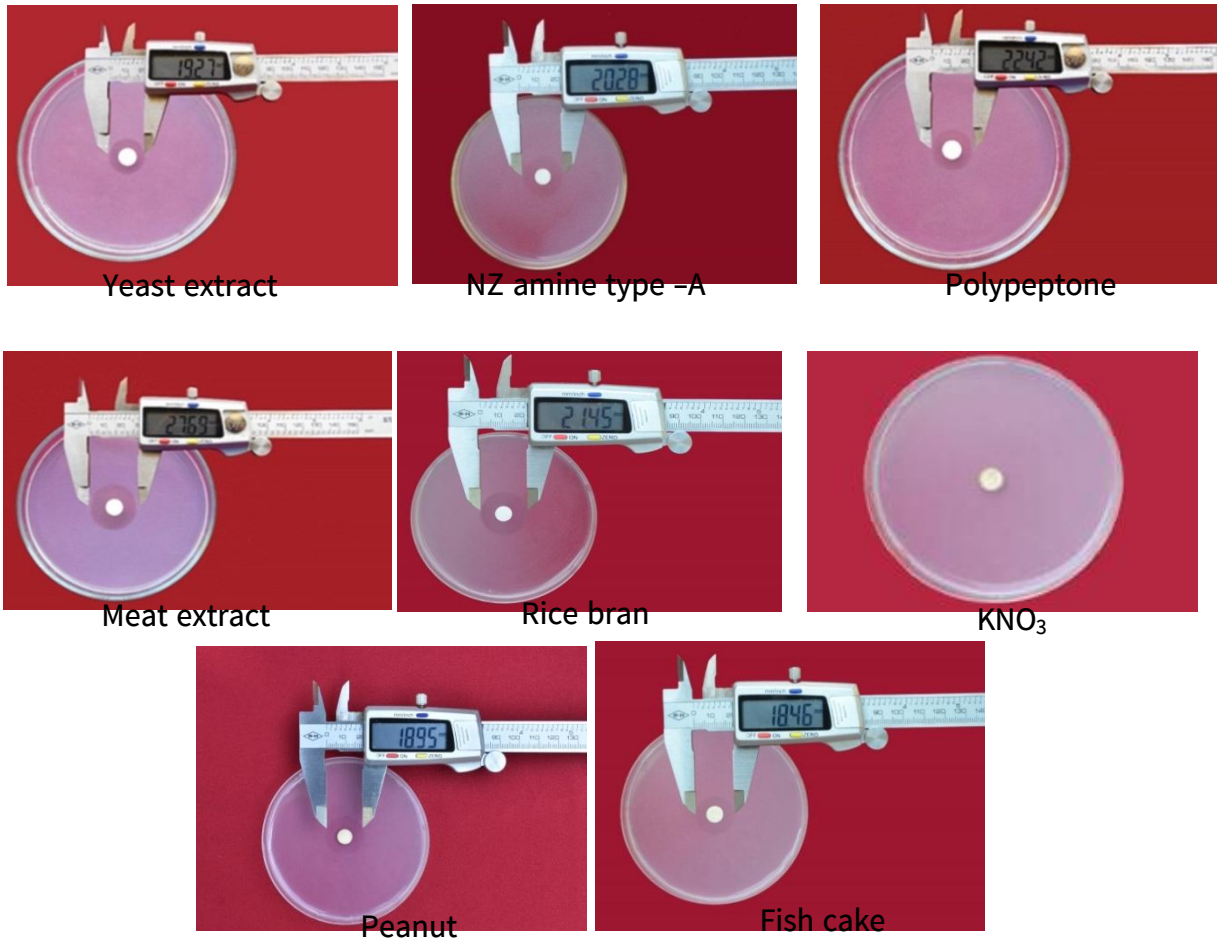


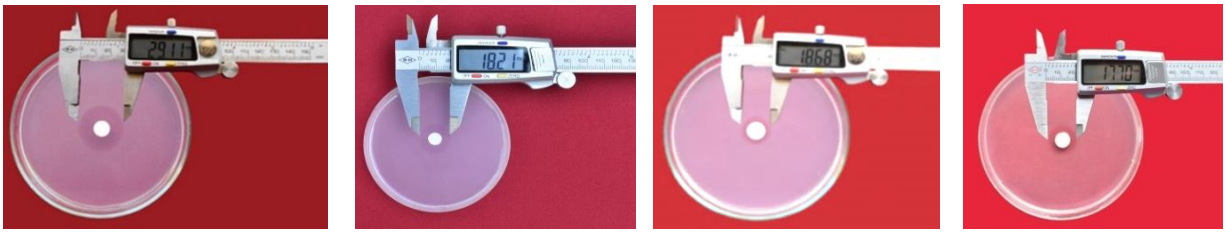
Figure 5. The effect of nitrogen sources on fermentation of fungus *Trichoderma reeseii*

Production of Antibacterial Metabolite from Four Different Fermented Media by Fungi *Trichoderma reeseii*

In the present study four kinds of fermentation media were used. According to the results of antibacterial activity, fermentation medium FM–1 showed the highest inhibitory zone of 29.11 mm, FM–2 showed the inhibitory zone of 18.21 mm, and FM–3 showed the inhibitory zone of 18.68mm , and FM–4 showed the inhibitory zone of 17.70mm (Table 5, Figure 6).

Table 5. Selection of medium based on the results of antibacterial activity (7 day fermentation)

Fermentation media	Inhibitory zone (mm)
FM–1	**29.11**
FM–2	18.21
FM–3	18.68
FM–4	17.70



FM–1 FM–2 FM–3 FM–4

Figure 6. The Antibacterial Activity of *Trichoderma reeseii* on Fermentation medium FM–1, FM–2, FM–3 and FM–4 (7 days fermentation)

Discussion and Conclusion

The choice of carbon and nitrogen sources greatly influenced in the production of secondary metabolism. Different carbon and nitrogen sources have been employed to be suitable for production of secondary metabolites (Stanbury, *et al*. 1997). In the present study, eight kinds of different carbon sources were used to observe the growth of selected fungus. The sources from soluble starch, molasses and potato powder showed the excellent growth. The sources from glucose, glycerol and Tapioca powder showed the good growth and sucrose and fructose showed the poor result (Table 1, Figure 1). Therefore, the best fermentation was soluble starch in the broth for carbon sources.

The nature of the nitrogen source used has a notable effect on the production of the antimicrobial metabolite. Moreover, eight kinds of nitrogen sources were also used to observe the growth of selected fungus. The sources from NZ–amine type A, meat extract, KNO_3, peanut and fish cake showed the excellent growth. The sources from yeast extract and polypeptone were showed the good growth. The source from rice bran was showed poor growth (Table 2, Figure 2). Therefore, the best fermentation was polypeptone, in the broth for nitrogen sources. According to the results from present study, it is concluded that the greatest age of culture is 84 hours and the optimum size of inoculum is 20%. Therefore, fermentation was undertaken with 20% size of inoculum and 84 hrs ages of culture (Figure 3).

In this research work, carbon and nitrogen containing media were investigated; carbon sources such as glucose, glycerol, soluble starch, molasses (Table 3, Figure 4) and nitrogen sources such as yeast extract, meat extract, N–Z amine type A, KNO_3, polypeptone, were showed excellent activity on *Escherichia coli* (Table– 4, Figure– 5).

The choice of a good fermentation medium is virtually as important to the success of an industrial fermentation as is the selection of an organism to carry out the fermentation (El–Tayeb, *et al.,* 2004). In this research work, four kinds of fermentation media were used for the antibacterial activity of *Trichoderma reeseii*. Among the four fermentation media, the medium– 1 (FM–1) showed the greater antibacterial activity than other (Table –5 and Figure–6). Therefore, fermentation medium–1 was selected for further investigation such as extraction and purification of antibacterial metabolite. Watt, *et al*., 1988, Thakur, *et al.,* 2009 reported that the *Trichoderma sp.* grew on all the carbon sources and nitrogen sources and

tested against bacterial pathogen and the maximum growth and bioactivity of the strain was noted. The results are in good agreement with Thakur, *et al.,* 2009.

These investigations clearly indicate that the selected fungus *Trichoderma reeseii* are useful for the production of antibacterial metabolite especially against *Escherichia coli* from fermentation medium–1.

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