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# Production of Pecticlyases by Three Thermophilic Fungi

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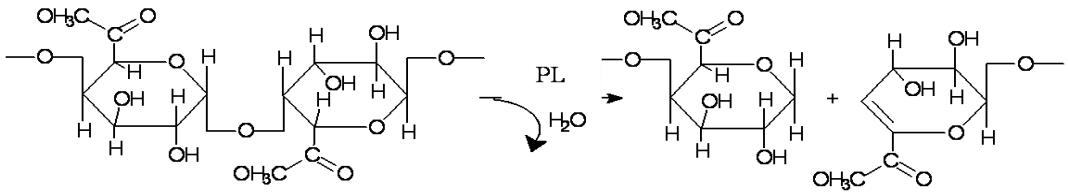
## ABSTRACT

**Production of pecticlyases by three thermophilic fungi, i.e *Thermomyces lanuginosus, Talaromyces luteus* and *Rhizomucor pusillus* under different cultural conditions was studied. Although all the three fungi were able to produce pecticlyases (exo-pectinlyases (exo-PL), endo-pectic acid lyase (endo-PAL), exo-pectinlyase (exo-PL) and pectic acid layse (endo-PAL) the degree of production varied with the fungus and prevailing environmental conditions. Temperature of 45ºC and pH 6.0-7.0 were optimum for production of different pecticlyases by the fungi understudy. The preference of carbon and nitrogen sources varied both with the fungus and enzyme concerned. A positive correlation could be observed between mycelial growth and pecticlyases production. The production of pecticlyases by all the three fungi under investigation proved to be adaptive.**

**Keywords:** Pecticlyase, *Thermomyces lanuginosus*, *Talaromyces luteus*, *Rhizomucor pusillus*, Exo- and endo-pectinlyase, Exo-and endo-pecticacidlyases

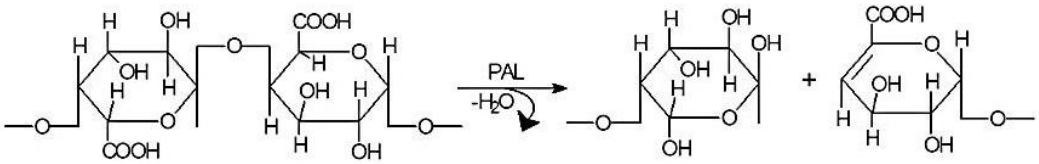
## INTRODUCTION

The pectic substances are a group of closely related polysaccharides form the primary cell wall and intercellular regions of higher plants. These are largely responsible for integrity and coherence of plant tissue (Wasby *et al.*, 1997). These consist of protopectin, pectin and pectic acid which are polymers of rhamnogalacturonic acid and are acted by group of enzymes known as pectinases. These pectinases or transeliminases are produced by microorganisms specially fungi and responsible for tissue disintegration and establishment of fungal pathogen resulting in the plant disease. Pecticlyases act on pectic substances and remove the proton of $C_5$ of one residue which results in the formation of unsaturated bond between $C_4$ and $C_5$ of polygalacturonic acid, thus resulting in unsaturated galacturonyl units (**Figure 1, Figure 2**). These unsaturated products have absorption maxima at 235/236nm and on treatment with thiobarbituric acid (TBA) gives an absorption maxima at 547 nm. These enzymes are classified on the basis of their preference to the substratum (pectin or pectic acid) and are named as pectinlyase (PL) or pectic acid lyase (PAL) respectively. Similarly, based on the mode of attack on Polygalcturonic acid chain randomly or in a sequential manner from one end of the chain are called endo-lyases or exo-lyases respectively.




**Pectin** **PMG**

**Figure 1:** Action of pectin transeliminase.




**Polygalacturonic acid** **Galacturonic acid**

**Figure 2:** Action of polygalacturonic acid transeliminase.

The active role of these transeliminases in host-pathogen interaction and cause of plant disease has been well established (Walton and Cervone, 1990; Adejuwan *et al.,* 2006; Jamile *et al.,* 2010). They are also extensively used in cannery industry, food processing, fruit juices and vegetable oils (Kashyap *et al.,* 2000), wine industry (Ashok and Ramachandran, 2005; Suryam *et al.,* 2018). Production of transeliminases by fungi has been reviewed by Satyanarayana *et al.* (2005). Though production of transeliminases by mesophilic fungi was studied (Sakamoto *et al.,* 1994; Suryam *et al.,* 2018), production of these enzymes by thermophilic fungi was studied only to a limited extent (Blanco *et al.,* 1999). Therefore, in the present investigations production of pecticlyases by three thermophilic fungi was studied. Factors influencing their production was also investigated and discussed in this communication.

## MATERIALS AND METHODS

Production of pecticlyases by three thermophilic fungi viz *Thermomyces lanuginosus, Talaromyces luteus* and *Rhizomucor pusillus* was studied in different culture media and environmental conditions. Monosporic cultures of above fungi were grown in 25 ml different media contained in 100 ml Erlenmeyer conical flask and inoculated with 0.5 ml spore suspension and incubated at 40 ºC for 8 days. The pH of the medium was adjusted to pH 5.5. At the end of the incubation period cultures were harvested in pre-weighted Whatman No.42 filter paper. The filter papers along with fungal mycelium were dried in an oven at 65 ºC for 48 h and cooled to room temperature in a desiccator for 24 h and weighted to a constant value in an analytical balance.

The culture filtrate thus obtained was centrifuged at x1800 for 30 min and dialysed against distilled water over night and served as an enzyme sample. Endo-pectinlyase (endo-PL) and endo-pectic acid lyase (endo-lyase) were assayed viscometrically. Fifteen ml of pectin (1%) or pectic acid (0.5%) solution, 1.0ml buffer and 5ml enzyme sample was taken in a Oswald-Fenske viscometer (size 150 with minimal efflux time of 15-20 seconds of water), mixed the contents and efflux time of the reaction mixture was recorded at 5 min. interval for 30 min. Reaction mixture with heat inactivated enzyme served as control. The enzyme activity was calculated in terms of loss of viscosity.

$$V = \frac{t_1 - t_a}{t_1 - t_o} \times 100$$

Where $t_1$ = initial flow of reaction mixture + inactive enzyme
$t_o$ = initial flow time of water + active enzyme
$t_a$ = Flow time of reaction mixture + active enzyme
V=percentage loss of viscosity

Percentage of loss of viscosity of substratum was calculated. The time required for 50% loss of viscosity was calculated by the formula and expressed as relative enzyme activity.

$$\text{Relative enzyme activity (RA)} = 1000 / t_{50}$$

Where $t_{50}$ is the time required for 50% loss of viscosity.

The activity of exo-pecticlyases was assayed by the method suggested by Sherwood (1966). The production of lyase was detected by the unsaturated product formed due to enzyme reaction, which has absorption maxima at 547 nm on reaction with thiobarbutaric acid. The substratum for exo-PAL was 0.5% Napp, while for exo-PL it was 1 % pectin. The reaction mixture consisting of 4 ml of substratum (pectin or pectic acid), 2 ml of enzyme and 1 ml of tris-HCl buffer (pH 8.1) was incubated at 30±1 ºC for 4 hours. At the end of incubation period, 1 ml of reaction mixture was withdrawn into a test tube and 5 ml of thiobarbutaric acid (TBA) was added followed by 1.25 ml of 1 N HCl and kept in hot water bath for one hour, cooled to room temperature and the absorbance was read in a range of 475-575 nm. A peak at 547 nm was considered as a positive for production of lyases. Lyase activity was expressed in IU (International unit). An increase in 0.01 OD was taken as one unit.

Effect of pH and temperature on production of pecticlyases by three thermophilic fungi was also studied by making suitable alterations. Effect of carbon and nitrogen sources on pecticlyase production was studied by substituting carbon and nitrogen sources by employing formula suggested by Mao *et al.,* (2005), so as to supply equal amount of carbon and nitrogen respectively in the medium.

$$\text{Amount of C/N in the medium} = \frac{\text{Molecular weight of new C/N sources}}{\text{12/14 x No. of C/N molecule present in the compound formula of new source}} \times \text{Amount of C/N required}$$

$$\text{Amount of C/N required} = \frac{12/14 \times \text{amount of C/N present in original medium}}{\text{Molecular weight of C/N source present in original composition of medium}} \text{ X Amount of C/N required}$$

## RESULTS AND DISCUSSION

Production of pecticlyases (exo- and endo-PAL) by three thermophilic fungi on different synthetic media was studied and the results are presented in **table 1**.

**Table 1** reveals that all the three thermophilic fungi could produce pecticacidlyase (PAL) in one or the other medium. However, the degree of production varied both with the organism and the medium. Medium C induced maximum amount of exo-PAL in all the three thermophilic fungi under investigation. Medium D and A were poor in induction of exo-PAL in *T.lanuginosus* and *T. luteus*. Rest of the media supported intermediate amount of exo-PAL production. Medium E followed by D were poor substrates for the production of exo-PAL by *R. pusillus*. Activity of exo-PAL increased with the increased amount of pectin added. Low production of exo-PAL in medium lacking pectin and comparatively more production of this enzyme in medium containing pectin suggests the adaptive nature of the exo-PAL of all the three fungi under investigation. Similarly *Penicillium citrinum* (Olutiola and Akintunde, 1979) and *Acrocylindrium oryzae* (Reddy *et al.*, 1985) are reported to produce exo-PAL adaptively.

**Table 1:** Production of PAL (exo*and endo**) and PL (exo*and endo**) production during 8 days incubation by three thermophilic fungi

| Medium | *T. lanuginosus* | | | | *T. luteus* | | | | *R. pusillus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PAL | | PL | | PAL | | PL | | PAL | | PL | |
| | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo |
| Yeast Extract Starch medium (A ) | 7.0 | 11.2 | 11.0 | 12.8 | 10.0 | 3.5 | 14.0 | 12.4 | 15.0 | 20.1 | 4.0 | 12.6 |
| Yeast Extract Starch +0.1 % Pectin (B) | 11.0 | 14.5 | 22.0 | 14.4 | 13.0 | 11.8 | 12.0 | 17.3 | 17.6 | 17.1 | 8.0 | 19.3 |
| Yeast Extract Starch +0.5% Pectin (C) | 12.0 | 19.4 | 25.0 | 18.3 | 18.0 | 19.2 | 19.0 | 18.2 | 19.0 | 22.8 | 10.0 | 21.2 |
| Yeast Extract Glucose (D) | 6.0 | 10.4 | 12.0 | 10.2 | 9.0 | 7.7 | 7.0 | 12.4 | 9.0 | 13.1 | 3.0 | 14.8 |
| Yeast Extract Glucose + 0.5% Pectin (E) | 9.0 | 12.5 | 14.0 | 14.2 | 12.0 | 11.7 | 13.0 | 13.8 | 8.0 | 14.7 | 5.0 | 14.7 |

*Expressed in units (0.01, O.D. change was taken as 1 unit of enzyme activity)
** Expressed in relative viscometric units (RVU)

All the three thermophilic fungi under study produced endo-PAL in one or other media tried. The endo-PAL production was maximum in medium C by all the present thermophilic fungi. Medium B and E were next preferred substrates for the production of endo-PAL by *T. lanuginosus* and *T. luteus*, while *R. pusillus* did the same in medium A and B.

Critical perusal of **table 1** reveals that the three thermophilic fungi under study could produce pectin lyase in one or other medium. However, the degree of production varied with the organism and the medium. Medium D and A were least preferred substrates for exo-PL production by all the present fungi. Medium C which contained pectin supported maximum exo-PL suggesting the adaptive nature of the exo-PL of all the three thermophilic fungi under study. Similarly Mehta *et al.*, (1993) have reported the adaptive nature of PL produced by *Aspergillus niger*.

Medium C followed by Medium B was ideal substratum for the production of endo-PL by *T. lanuginosus*, while medium E and A induced only meager amount of endo-PL. *T. luteus* could produce endo-PL in almost all the media tried with slight variation in the degree of production. Endo-PL production by *R. pusillus* was maximum in medium C, while Medium B and E were next preferred substrates for the production of endo-PL.

Influence of pH on PAL (exo- and endo-) and PL (exo- and endo-) production by three thermophilic fungi was studied and the results are précised in **table 2**.

**Table 2:** Effect of pH on PAL (exo*and endo**) and PL (exo*and endo**) production during 8 days incubation by three thermophilic fungi

| pH | *T. lanuginosus* | | | | *T. luteus* | | | | *R. pusillus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PAL | | PL | | PAL | | PL | | PAL | | PL | |
| | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo |
| 4.0 | 2.0 | 6.6 | 5.0 | 5.5 | -- | -- | -- | -- | 2.0 | 11.1 | 1.0 | 11.8 |
| 5.0 | 1.0 | 8.1 | 9.0 | 8.8 | 1.0 | 4.6 | 1.0 | 8.2 | 7.0 | 12.9 | 3.0 | 14.2 |
| 6.0 | 7.0 | 17.1 | 11.0 | 12.8 | 10.0 | 3.5 | 14.0 | 10.4 | 15.0 | 20.1 | 4.0 | 12.6 |
| 7.0 | 3.0 | 10.2 | 11.0 | 9.1 | 7.0 | 12.1 | 10.0 | 18.9 | 2.0 | 11.8 | 5.0 | 11.9 |
| 8.0 | 3.0 | 2.8 | 6.0 | 6.0 | 4.0 | 11.7 | 5.0 | 11.2 | 6.0 | 7.3 | 2.0 | 11.9 |
| 9.0 | -- | -- | -- | -- | 18.0 | 12.1 | 36.0 | 18.2 | 7.0 | 11.2 | 3.0 | 16.2 |

*Expressed in units (0.01, O.D. change was taken as 1 unit of enzyme activity)
** Expressed in relative viscometric units (RVU)

Exo-PAL production by *T. lanuginosus* and *R. pusillus* was maximum at pH 6.0, while no exo-PAL production could be recorded at pH 9.0 (**Table 2**). *T. luteus* failed to produce exo-PAL at pH 4.0. *T. luteus* produced good amount of exo –PAL even at pH 9.0. Chary and Reddy (1983) have also recorded maximum production of PAL by *Phoma exigue* under alkaline condition (pH 9.0). The exo-PAL production by *T. lanuginosus* and *R. pusillus* decreased gradually with the increase or decrease in alkalinity. Similar trend was observed in endo-PAL production.

Maximum amount of exo-PL was secreted by the all the fungi under investigation at pH 6.0 which decreased with increasing and decreasing alkalinity. The exo-PL production by *T. lanuginosus* was inhibited at pH 9.0. *T. luteus* was inhibited for enzyme production at pH 4.0, while *R. pusillus* produced good amount of exo-PL at pH 7.0.

*T. lanuginosus* recorded maximum production of endo-PL at pH 6.0 (**Table 2**). No endo-PL production could be recorded at pH 9.0. *T. luteus* produced good amount of endo-PL at pH 7.0 and it failed to produce at pH 4.0. *R. pusillus* opted pH 9.0 for the maximum production of endo-PL, while it was low at pH. 4.0. Khairnar *et al*., (2009) have reported maximum activity of pectin lyase of *Aspergillus niger* at pH 6.6.

Effect of temperature on PAL (exo- and endo-) and PL (exo-and endo) production by three thermophilic fungi was investigated and the results are tabulated in **table 3**.

From **table 3** it is clear that the exo-PAL production by all the three thermophilic fungi was maximum at incubation temperature of 45 ˚C. Further increase or decrease in incubation temperature resulted in decreased enzyme production. No exo-PAL production by *R. pusillus* could be recorded during early phase of incubation period at 35 ˚C. At an incubation temperature of 55 ˚C the exo-PAL production by *R. pusillus* ceased. Exo-PL production by all the three fungi was maximum at incubation temperature of 45 ˚C and decreased both with increase or decrease of incubation temperature. The endo-PAL production by all the three thermophilic fungi was maximum at 45 ˚C. Its production decreased with the increase or decrease in incubation temperature. Almost same trend was observed with the production of Endo-PL. *R. pusillus* ceased the production of endo-PL at incubation temperature of 55 ˚C.

Influence of carbon source on the production of PAL (exo- and endo-) and PL (exo- and endo-) by three thermophilic fungi was studied and the results are summarized in **table 4**.

It is clear from **table 4** that the type of carbon source present in the medium had profound influence on the production of PAL by the fungi under investigation. Such specificity of fungi towards carbon source for exo-PAL production was also reported by Dube and Bordia (1982) and Teixeira *et al.,* (2000). Mannitol followed by glycerol, sucrose and starch were the good carbon sources for the production of exo-PAL by *T. lanuginosus*, while D-glucose, lactose, succinic acid were poor inducers of exo-PAL.

**Table 3:** Effect of temperature on PAL (exo*and endo**) and PL (exo*and endo**) production during 8 days incubation by three thermophilic fungi

| Tempe-rature (in $^{o}$C) | *T. lanuginosus* | | | | | *T. luteus* | | | | | *R. pusillus* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dry wt. (in µg) | PAL | | PL | | Dry wt. (in µg) | PAL | | PL | | Dry wt. (in µg) | PAL | | PL | |
| | | Exo | Endo | Exo | Endo | | Exo | Endo | Exo | Endo | | Exo | Endo | Exo | Endo |
| 35 | 155.4 | 1.0 | 7.6 | 1.0 | 4.6 | 132.3 | 3.0 | 5.5 | 3.0 | 6.8 | 192.2 | 2.0 | 5.5 | 2.0 | 3.6 |
| 40 | 187.6 | 9.0 | 17.2 | 6.0 | 16.8 | 177.2 | 8.0 | 14.6 | 5.0 | 14.3 | 238.4 | 6.0 | 16.2 | 1.0 | 20.6 |
| 45 | 212.2 | 10.0 | 24.2 | 9.0 | 23.4 | 185.2 | 11.0 | 23.6 | 10.0 | 22.2 | 275.5 | 9.0 | 25.3 | 4.0 | 25.6 |
| 50 | 256.3 | 9.0 | 16.2 | 10.0 | 19.4 | 178.5 | 7.0 | 16.5 | 9.0 | 20.2 | 270.2 | 6.0 | 18.3 | 3.0 | 24.6 |
| 55 | 255.3 | 2.0 | 11.2 | 2.0 | 14.2 | 133.2 | 7.0 | 13.1 | 7.0 | 14.2 | 210.2 | -- | -- | -- | -- |

*Expressed in units (0.01, O.D. change was taken as 1 unit of enzyme activity)
** Expressed in relative viscometric units (RVU)

*T. lanuginosus* and *T. luteus* failed to produce exo-PAL in medium containing citric acid. *T. luteus* opted D-glucose and D-fructose for the maximum production of exo-PAL, while succinic acid and lactose were poor substrates for the production of exo-PAL. Rest of the carbon sources induced varying amount of exo-PAL. Mannitol, glycerol and starch could induce maximum exo-PAL in *R. pusillus*, while citric acid, succinic acid and D-fructose were the poor substrates for the production of exo-PAL. Rest of the carbon sources induced intermediate amount of exo-PAL in *R. pusillus*.

In contrast to exo-PAL production, endo-PAL production was comparatively uniform in different carbon source present in the medium. Sucrose followed by mannitol and D-fructose induced maximum endo-PAL in *T. lanuginosus*, while succinic acid and lactose were poor substrates for endo-PAL production. D-fructose followed by D-glucose were responsible for maximum production of endo-PAL by *T. luteus*, while lactose, succinic acid and L-sorbose were poor substrates. Rest of the carbon sources supported moderate amount of endo-PAL activity in *T. luteus*. *R. pusillus* could produce maximum endo-PAL in the presence of mannitol and glycerol, while citric acid, succinic acid and lactose were poor inducers of endo-PAL. *R. pusillus* responded to rest of the carbon sources to an intermediate degree of endo-PAL production.

Mannitol followed by sucrose and D-fructose were the best sources for the production of exo-PL by *T. lanuginosus*, while citric acid totally inhibited the exo-PL production by *T. lanuginosus* and *T. luteus* (**table 4**). Succinic acid and lactose were poor carbon source for the production of exo-PL by *T. lanuginosus*. D-fructose and sucrose induced maximum of exo-PL activity in *T. luteus*, while D-glucose and L-sorbose were poor inducers of exo-PL.

*R. pusillus* opted mannitol and starch followed by glycerol for the maximum production of exo-PL, while D-glucose, D-fructose and sucrose were poor inducer for exo-PL.

D-fructose induced maximum endo-PL in *T. luteus*, while lactose was the poor substratum for endo-PL production. Rest of the carbon sources induced varying amount of endo-PL. Mannitol was the best source of carbon for *R. pusillus* for production of endo-PL, while, citric acid was the poor source of carbon. Rest of the carbon sources induced intermediate amount of endo-PL in *R. pusillus*. *T. lanuginosus* opted for mannitol followed by sucrose and D-fructose for elaboration of endo-PL, while succinic acid was a poor substratum for its elaboration.

**Table 4:** Influence of different Carbon sources on PAL (exo*and endo**) and PL (exo*and endo**) production during 8 days incubation period by three thermophilic fungi

| Carbon source | *T. lanuginosus* | | | | *T. luteus* | | | | *R. pusillus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PAL | | PL | | PAL | | PL | | PAL | | PL | |
| | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo |
| D-glucose | 7.0 | 16.3 | 6.0 | 15.7 | 29.0 | 27.2 | 6.0 | 19.5 | 8.0 | 21.2 | 4.0 | 12.4 |
| D-fructose | 10.0 | 24.2 | 9.0 | 23.4 | 29.0 | 28.3 | 17.0 | 34.2 | 7.0 | 21.4 | 6.0 | 17.3 |
| D-galactose | 11.0 | 14.2 | 12.0 | 12.0 | 18.0 | 14.6 | 10.0 | 18.3 | 21.0 | 19.8 | 13.0 | 19.3 |
| D-mannose | 18.0 | 13.2 | 14.0 | 19.5 | 18.0 | 14.0 | 5.0 | 17.9 | 17.0 | 22.9 | 7.0 | 18.3 |
| L-sorbose | 12.0 | 11.3 | 7.0 | 12.8 | 12.0 | 10.2 | 8.0 | 10.0 | 15.0 | 15.9 | 15.0 | 14.8 |
| D-xylose | 15.0 | 14.4 | 14.0 | 12.5 | 15.0 | 13.2 | 12.0 | 13.2 | 14.0 | 19.2 | 14.0 | 17.3 |
| Sucrose | 22.0 | 29.8 | 21.0 | 24.9 | 19.0 | 22.8 | 16.0 | 26.8 | 10.0 | 19.6 | 6.0 | 15.8 |
| Maltose | 18.0 | 13.7 | 15.8 | 21.8 | 22.9 | 13.2 | 12.3 | 18.2 | 18.0 | 21.2 | 11.0 | 19.2 |
| Mannitol | 30.0 | 28.2 | 18.0 | 33.1 | 22.0 | 24.8 | 12.0 | 28.5 | 38.0 | 56.4 | 23.0 | 33.5 |
| Citric acid | -- | -- | -- | -- | -- | -- | -- | -- | 4.0 | 5.5 | 5.0 | 6.6 |
| Succinic acid | 8.0 | 8.2 | 9.0 | 6.6 | 5.0 | 9.4 | 8.0 | 14.2 | 5.5 | 9.1 | 11.0 | 10.4 |
| Lactose | 8.0 | 9.7 | 9.0 | 12.2 | 12.3 | 9.5 | 10.0 | 9.7 | 12.0 | 13.2 | 11.0 | 13.6 |
| Glycerol | 22.0 | 18.5 | 16.0 | 16.4 | 19.0 | 18.5 | 11.0 | 18.4 | 21.0 | 28.6 | 17.0 | 22.6 |
| Starch | 21.0 | 11.5 | 23.0 | 12.2 | 16.0 | 11.9 | 20.0 | 15.6 | 23.0 | 12.4 | 26.0 | 14.8 |

*Expressed in units (0.01, O.D. change was taken as 1 unit of enzyme activity)
** Expressed in relative viscometric units (RVU)

Citric acid failed to support the growth and endo-PL production by *T. lanuginosus*. Rest of the carbon sources supported intermediate degree of endo-PL production.

Reaction of three thermophilic fungi towards different nitrogen sources for the production of PAL (exo- and endo-) and PL (exo- and endo-) was studied and the results are depicted in **table 5**.

**Table 5** reveals that the three thermophilic fungi exhibited specificity towards nitrogen source present in the medium. *T. lanuginosus* and *T. luteus* could produce good amount of exo-PAL in the presence of L-asparagine and L-alanine, while L-methionine and L-tyrosine induced limited exo-PAL production. Rest of the nitrogen sources induced intermediate degree of exo-PAL in *T. lanuginosus*. Ammonium nitrate, L-cystine and L-aspartic acid were poor inducer of exo-PAL in *T. luteus*. L-arginine, L-glycine and L-glutamic acid were the good substrates for the production of exo-PAL by *R. pusillus*. *R. pusillus* was comparatively superior in production of exo-PAL than other two fungi under investigation. The exo-PAL production was very low in medium containing ammonium nitrate, L-cystine and ammonium chloride.

L-arginine, L-alanine, L-glycine and yeast extract were good nitrogen sources for the production of endo-PAL by *R. pusillus*, while ammonium sulphate was poor nitrogen source. Rest of the nitrogen sources were moderate nitrogen sources for the induction of endo-PAL. *T. luteus* opted L-asparagine for the production of endo-PAL. Endo-PAL production by *T. luteus* was least in medium containing L-methionine, L-tryptophan and L-tyrosine, while, rest of the nitrogen sources induced intermediate amount of endo-PAL. *T. lanuginosus* produced good amount of endo-PAL in medium containing L-tyrosine, L-tryptophan, L-methionine and yeast extract, while ammonium sulphate, ammonium nitrate, L-cystine and L-lysine were responsible for low enzyme activity. Rest of The nitrogen sources supported moderate amount of endo-PAL. L-glycine and L-alanine were best nitrogen sources for the production of exo-PL by *T. lanuginosus*, while ammonium sulphate, L-cystine, L-aspargine and L-aspartic acid were poor substrates for the activity of exo-PL. *T. luteus* produced maximum exo-PL in the presence of L-histidine, L-aspargine and L-alanine, while L-aspartic acid and L-cystine were poor inducers of exo-PL in *T. luteus*.

**Table 5:** Influence of different nitrogen sources on PAL (exo*and endo**) and PL (exo*and endo**) production during 8 days incubation period by three thermophilic fungi

| Nitrogen source | *T. lanuginosus* | | | | *T. luteus* | | | | *R. pusillus* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PAL | | PL | | PAL | | PL | | PAL | | PL | |
| | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo | Exo | Endo |
| Ammonium chloride | 3.0 | 14.0 | 6.0 | 14.2 | 4.0 | 15.1 | 4.0 | 14.8 | 3.0 | 14.6 | 8.0 | 16.2 |
| Ammonium nitrate | 4.0 | 15.2 | 7.0 | 12.4 | 1.0 | 12.3 | 2.0 | 14.5 | 1.0 | 13.7 | 5.0 | 14.8 |
| Ammonium sulphate | 1.0 | 11.1 | 1.0 | 12.1 | 4.0 | 9.1 | 2.0 | 12.3 | 3.0 | 6.2 | 2.0 | 13.2 |
| L-alanine | 7.0 | 19.2 | 12.0 | 17.2 | 7.0 | 18.4 | 8.0 | 17.1 | 9.0 | 23.1 | 12.0 | 22.1 |
| L-arginine | 6.0 | 18.3 | 7.0 | 12.8 | 5.0 | 19.4 | 4.0 | 18.2 | 13.0 | 24.5 | 12.0 | 22.3 |
| L-aspargine | 9.0 | 18.4 | 11.0 | 19.4 | 9.0 | 21.3 | 8.0 | 22.2 | 9.0 | 18.8 | 6.0 | 17.3 |
| L-aspartic acid | 3.0 | 11.2 | 3.0 | 11.5 | 1.0 | 9.0 | 1.0 | 9.3 | 9.0 | 16.3 | 11.0 | 19.2 |
| L-glutamine | 4.0 | 21.4 | 12.0 | 17.8 | 5.0 | 17.8 | 4.0 | 17.7 | 7.0 | 19.1 | 4.0 | 18.6 |
| L-glutamic acid | 2.0 | 15.0 | 9.0 | 16.2 | 4.0 | 16.4 | 8.0 | 15.3 | 11.0 | 19.1 | 11.0 | 16.4 |
| L-Glycine | 6.0 | 18.9 | 13.0 | 18.5 | 7.0 | 24.4 | 9.0 | 18.2 | 12.0 | 22.2 | 11.0 | 22.2 |
| L-methionine | 3.0 | 19.1 | 7.0 | 13.2 | 4.0 | 9.1 | 2.0 | 12.3 | 3.0 | 19.1 | 7.0 | 13.2 |
| L-cystine | 4.0 | 9.1 | 2.0 | 12.3 | 1.0 | 11.1 | 1.0 | 12.1 | 3.0 | 14.6 | 8.0 | 16.2 |
| L-histidine | 3.0 | 14.0 | 6.0 | 14.2 | 3.0 | 14.6 | 8.0 | 16.2 | 4.0 | 16.5 | 6.0 | 16.7 |
| L-lysine | 4.0 | 9.1 | 2.0 | 12.3 | 4.0 | 15.1 | 4.0 | 14.8 | 3.0 | 6.2 | 2.0 | 13.2 |
| L-tryptophan | 4.0 | 20.0 | 6.0 | 15.2 | 4.0 | 9.1 | 2.0 | 12.3 | 8.0 | 18.4 | 5.0 | 17.2 |
| L-tyrosine | 3.0 | 19.1 | 7.0 | 13.2 | 1.0 | 9.0 | 1.0 | 9.3 | 4.0 | 18.7 | 2.0 | 16.6 |
| Yeast Extract | 4.0 | 15.2 | 10.0 | 22.2 | 6.0 | 17.2 | 4.0 | 19.5 | 9.0 | 23.2 | 8.0 | 17.2 |

*Expressed in units (0.01, O.D. change was taken as 1 unit of enzyme activity)
** Expressed in relative viscometric units (RVU)

L-alanine followed by L-arginine, L-glutamic acid and L-glycine were best nitrogen sources for the production of exo-PL by *R. pusillus*, while *L*-lysine, L-tryptophan and L-tyrosine were poor nitrogen sources. Rests of the nitrogen sources were intermediate in their efficiency in supporting exo-PL production by *R. pusillus*.

Endo-PL production by *T. lanuginosus* was maximum in medium containing L-tyrosine, L-methionine and L-tryptophan, while other nitrogen sources were inferior in their efficiency in induction of endo-PL. *T. luteus* opted L-aspargine, yeast extract and L-arginine for maximum production of endo-PL. L-aspartic acid and L-tyrosine were poor inducers of endo-PL in *T. luteus*. Rest of the nitrogen sources induced intermediate amount of endo-PL. The endo-PL production by *R. pusillus* was maximum in medium containing L-arginine, L-glycine and L-alanine nitrogen sources, while all other nitrogen sources screened in this study supported low amount of endo-PL activity.

## CONCLUSION

Present investigations reveal the good potential of three thermophilic fungi under study for production of pecticlyases. Pecticlyase of exo-PAL and endo-PAL production by all the three thermophilic fungi was maximum at 45˚C. The degree of production of different pecticlyases varied with the fungus and environment under which the fungus was growing. Production of pecticlyases by the fungi under investigation was found to be adapted.

## CONFLICT OF INTEREST

The authors hereby declare that they have no conflict of interest

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