EFFECT OF CHICKPEA LEAF LEACHATES, SAFFLOWER LEAF LEACHATES, NACl TREATMENTS AND PLANT GROWTH REGULATORS TREATMENT AND PEG TREATMENTS ON LIPASE ACTIVITY IN DIFFERENT *Linum usitatissimum* VARIETIES DURING GERMINATION

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ABSTRACT

Linseed (*Linum usitatissimum* L.) is an oil seed crop and is taken as a marginal as well as intercrop between chickpea and safflower in many parts of Solapur district which is normally a drought prone area. Generally, it is well known fact that enzyme lipases plays a key role during seed germination. Hence here in the present investigation, an attempt has been made to study effect of Chickpea leaf leachates, Safflower leaf leachates, NaCl treatments and Plant Growth Regulators treatment and PEG treatments on lipase enzyme activity in different *Linum usitatissimum* varieties during germination. It was noticed that the activity of lipase was found to be reduced due to PEG induced water stress, in all the three varieties while salt stress was found to cause marked inhibition of enzyme lipase in Local and Nagarkot varieties, chickpea and safflower leaf leachates adversely affect the lipase activity at 5% concentration while lipase enzyme activity was found to be enhanced due to all PGR treatments.

KEYWORDS: Linseed, salinity, water stress, lipase activity, leachates.

INTRODUCTION

Lipase is also known as triacylglycerol acyl hydrolase. Lipase is known to be present in oil bodies of oil seeds (Huang et al., 1983). Lipases has vital applications in various industries such as the food, pharmaceutical, fine chemical, oil chemical, biodiesel and industrial
detergent (Freire and Castilho 2008, Alonso et al. 2005). Lipases play vital roles in the digestion, transport and processing of dietary lipids in living organism. According to Macrae and Hammond (1985) and Sonnet (1988) and Villeneuve (2003), lipase specificities can be divided into three main groups as Substrate specificity, Regioselective & Enantioselective. They are able to catalyze the hydrolysis of triacylglycerols (TAGs) as well as di- and monoacylglycerols and even phospholipids and has ability to discriminate enantiomers in a racemic mixture. Enujiugha (2007) reported lipases produced by animals, plants and microorganisms helps to hydrolyze triacylglycerols into fatty acids and glycerol. It also catalyses the hydrolysis of various forms of fatty acyl esters and in contrast to other esterases needs an oil-water interface for its optimum activity. Grains generally contain proteins and, depending on the plant species, starch or triacylglycerols as energy reserve sources. From 20% to 50% of the dry weight of oilseeds is basically stored triacylglycerols. Lipase hydrolyses only carboxyl ester bonds therefore they are considered as carboxyl esterase (Sonnet, 1988). Lipase requires a high pH for its activation among food enzymes. Most of the nutrients available are hydrolyzed specifically by proteases, amylases and lipases, respectively. Lipases has important role in physiological functions during storage of various agricultural products. Many researchers have investigated seed lipase at the height of its activity during grain germination (Hellyer et al., 1999; Parques and Macedo, 2006). In the present study we report the effect of Chickpea leaf leachate, Safflower leaf leachate, NaCl, PGR and PEG on lipase activity of three different varieties of *Linum usitatissimum* (Local variety, NL97- Variety, Nagarkot variety).

**MATERIALS AND METHODS**

**Seed collection and germination**

Seeds of *Linum usitatissimum* Linn. (Flaxseed), Local variety was obtained from Sangola, NL-97 and Nagarkot varieties from the Agriculture College Nagapur and Chandra-Shekhar Azad Agriculture college, U. P. respectively. The seeds of these varieties were cleaned and used for further experiments.

**Lipase assay**

The flax seeds of all the three varieties were washed with water and blot to dryness. 10 g seeds were used for enzyme extraction. The oil from the seeds was removed by repeated extraction with cold petroleum ether. Each time the extract was filtered through double-layered muslin cloth. The seed powder free from oil served as the enzyme source.
Lipase activity was determined following the method of Wolf (1968). The five clean test tubes containing the reaction mixture 0.5ml Caster oil, 5ml phosphate buffer and 0.5 g enzyme source, were incubated at 37° c with constant shaking. The reaction was terminated at the end of the fixed time such as 0, 30, 60, 90 and 120 minutes by transferring the reaction mixture to a mixture of 30 ml ethanol and 10 ml petroleum ether. The content of the flasks was titrated against N/50 alcoholic KOH using phenolphthalein as an indicator. The end Point will be colorless to pink.

RESULTS AND DISCUSSION
The effect of Chickpea leaf leachate (cp), Safflower leaf leachate (SF), NaCl and PEG treatments on lipase activity of three different varieties of *Linum usitatissimum* (Local variety, NL-97 Variety, Nagarkot variety) has been reported in Table 1 and results have been depicted in Fig.1-5. The overall activity of enzyme lipase was highest (2.6 ml alkali h\(^{-1}\), g\(^{-1}\) fresh tissue) in germinating seeds of Local variety as compared to other NL-97 and Nagarkot varieties.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations</th>
<th>Local*</th>
<th>NL-97 *</th>
<th>Nagarkot *</th>
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<td>1.74</td>
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* ml alkali h\(^{-1}\), g\(^{-1}\) fresh tissue.

Table 1: Effect of various treatments on lipase activity in three different variety of *Linum usitatissimum*. 
The Chickpea leaf leachate treatment result in decline in lipase content at all concentrations (1% cp, 2% cp and 5% cp) as compared to control one. In Local variety it is up to 81.15% loss at 2% cp concentration as compared to other concentrations i.e. 57.69% decline at 1% cp and 79.23% decline at 5% cp concentration. It is evident that chickpea leaf leachate was responsible for reduction in overall activity of enzyme lipase. In case of NL-97 variety of *Linum usitatissimum*, 1% cp (Chickpea leaf leachate concentration) produces enhancing effect of the lipase (3.17%). While 2% cp and 5% cp both concentrations showed decline in lipase as compared to control. But as compared to Local variety, NL 97 variety showed higher tolerance to Chickpea leaf leachate treatments. In this variety maximum loss is up to 31.75% at 5% cp treatment and 20% loss at 2% cp treatment was evident. The fluctuation in lipase activity of Nagarkot variety of flaxseed showed similar trend as that of Local variety of flax i.e. decline in lipase content at all concentration. The highest decline in lipase was observed at 5% cp (loss 85.06%) as compared to other concentrations and controlled. The 65.52% loss and 13.79% decrease in lipase activity at 2% cp and 1% cp treatment respectively was evident.

Safflower leaf leachate treatment (SF) also showed similar results as that of chickpea leaf leachet treatment in varieties of *Linum usitatissimum*. i.e. the reduction in lipase activity at all SF concentrations in seeds of all varieties of *Linum usitatissimum* was evident. All varieties of *Linum usitatissimum* (Local, NL-97 & Nagarkot variety) showed maximum decline at 5% SF concentration. The highest 92.31% reduction in lipase activity was observed in Local variety at 5% concentration as compared to NL-97(84.13%) and Nagarkot variety (77.01%) reduction. At 1% SF and 2% SF concentration both, NL-97 variety showed maximum leachate tolerance as compared with other flaxseed varieties.
The NACL salinity treatments showed negative impact on lipase activity in all studied varieties of *Linum usitatissimum*. In present investigation effect of 0.4% NACL, 0.8% NACL and 1.2% NACL treatments showed 41.54%, 48.46% and 53.85% reduction in lipase activity in Local variety. In NL-97 variety of Linum, 12.70%, 19.05% and 25.40% reduction in lipase activity was observed under 0.4% NACL, 0.8% NACL and 1.2% NACL treatments respectively. In case of Nagarkot variety of Linum, 14.34%, 27.59% and 42.53% inhibition in lipase activity was observed under 0.4% NACL, 0.8% NACL and 1.2% NACL treatments respectively. It can also be concluded that salinity adversely affect the lipase activity, while among all studied varieties, NL-97 variety was found more tolerant to salinity than other studied varieties.

The Ascorbic acid treatment showed highest increase in lipase in NL-97 and Nagarkot variety. Ascorbic acid treatment for Local variety showed stable lipase activity. The GA treatment found highly beneficial to enhance lipase activity up to 163.85% increase and vipul treatment showed increase in lipase level up to 159.23% and CCC treatment found increased up to 148.46% in Local variety. The ascorbic acid and vipul treatment increases lipase up to
142.86% while CCC treatment results in 69.84% increase. While lowest increase in lipase activity was observed due to GA treatment (65.08%) in NL-97 variety. In Nagarkot variety, ascorbic acid treatment found highly effective than other PGR treatments (164.32% increase) and GA (34.48%), Vipul (18.39%) and CCC as 14.94% enhancement in lipase activity.

![Fig. 4 Effect of PGR treatments on lipase activity in three different varieties of Linum usitatissimum](image)

The all PEG treatments, Lipase get reduced. At the concentrations of PEG from 5 bar to 10 bar lipase activity was declined continuously as compared to control. The highest decline in lipase activity was observed in Local variety of flaxseed (61.54% reduction at 10 bar PEG treatment). While lowest decline was observed at 5 bar treatment (15.87%) in NL-97 variety. The Local variety showed highly susceptible to drought induced by PEG. Among all studied varieties Nagarkot variety showed less inhibition in lipase activity at 10 bar PEG treatment (31.03% decline) followed by NL-97 variety i.e. 32.54% reduction.

![Fig. 5 Effect of PEG induced waterstress treatments on lipase activity in three different varieties of Linum usitatissimum](image)

Black and Altschul (1965) observed a 2.5 fold increase in the lipase activity after treatment of the distal halves of cotton seeds with gibberellic acid. Bernmer and Hammond (1970) found that the lipase from oat showed preference for splitting fatty acids in 1 and 3 positions of the
triacylglycerol and the hydrolysis of natural fats was nonspecific but showed activity towards long chain fatty acid ester. Due to various potential lipase have been presently used in the various manufacturing processes of industrial goods such as detergent industry, food industry and in pharmaceutical industry (Boland et al., 1991; Gandh, 1997; Savendsen, 2000 and Bayoumi et al., 2007). Plant lipases may act as interesting substitutes for microbial and animal lipases in biotransformation reactions (Fahmy, et al., 1983 and Palocci et al., 2003). Lipase activity has been reported in several oil plant extracts which include castor bean, palm seeds, oleifera seed, sunflower seed, and peanuts (Abigar et al., 1985; Haderson and Osborne, 1991; Khan et al., 1991; Afolabi et al., 1991; Teirssene et al., 1995; Hoppe and Theimer 1997). Lipase is the primary digestant used to split fats into fatty acids and glycerol. Deficiency of lipase responsible towards high cholesterol, high triglycerides, difficulty losing weight and diabetes. Lipase deficient people have decreased cell permeability, meaning nutrients cannot get in and the waste cannot get out. Lipase modulates cell permeability so that nutrients can enter and wastes exit.

(http://www.enzymeessentials.com/HTML/lipase.html). The lipase inhibitors from various plant species have been investigated and reported, including Cassia nomame (Yamamoto et al., 2000), soybean seed (Satouchi et al., 1998), tea saponins (Birari and Bhutani, 2007), tea polyphenols (Nakai et al., 2005), grape seed (Moreno et al., 2003), Eriochloa villosa, Orixa japonica & Setaria italica (Sharma et al., 2005) and Platycodon grandiflorum (Han et al., 2000).

Basra et al. (2005) studied effect of Moringa extract on flax seed germination and reported that the extract treatment led to better germination flax and other crop plants also. Bousquet-Mélou et al. (2005) noted aqueous extracts of Medicago arborea strongly lowered the germination of yellow flax seeds. Zhao et al. (2010) studied allelopathy of aqueous extracts of Kochia scoparia and its different parts on flax (Linum usitatissimum). During this study they noticed that stem and leaf aqueous extracts of K. scoparia at different concentration shows different degree’s inhibition on flaxseed germination and seedling growth. Hesabi et al. (2014) reported germination behaviour of flax seeds under extract of yellow yarrow (Achillea santolina). They mentioned that aqueous extract of plant residues of yellow yarrow causes an increase of germination percentage in flax.

It is evident from result that among studied varieties highest lipase activity observed in Local variety (equivalent to 2.6 ml alkali h⁻¹. g⁻¹ fresh tissue) than that in other. In all studied flax
varieties in all treatment there is continuous fall down of lipase activity along with increasing treatment concentrations. Safflower leaf leachate treatment adversely effect on lipase activity in seeds of Local variety and NL-97 flax variety. While in Nagarkot variety lipase activity is highly decreases at 5% concentration of Chickpea leaf leachate treatment. In NaCl treatment higher decrease observed in local variety 1.2% than other varieties. At PEG induced water stress there is higher loss of lipase activity is observed at 10 bar in all varieties. It can be concluded from result that all studied treatment studied are non useful for increasing lipase activity.

CONCLUSION
From overall findings of current investigation it can be concluded and suggested that all PGR treatments are beneficial to farmer during cultivation of flaxseed varieties and suggested to treat them with ascorbic acid at the time of sowing. 5% Chickpea leaf leachate concentration observed toxic during seed germination in all varieties of Linum usitatissimum. Therefore avoid alternate planting flaxseed and chickpea, keep them away from each other. Nagarkot and NL-97 varieties are preferable to cultivation because it is observed that these varieties showed less fluctuation in their lipase under PEG induced drought stress. NL-97 variety found more tolerant for lipase inhibition under saline condition than other studied varieties. So this variety can be aid for saline land restoration or bioremediation as phytoremediation tool.

REFERENCES
