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Biostimulatory Effect of Shilajeet on Wheat (*Triticum astivum*) Seed Germination

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Abstract

A presoaking treatment of Shilajeet at three different concentrations (10, 100 and 1000ppm) was given for 8 hours to surface sterilized (with 0.1 % $HgCl_2$) seeds of wheat (Lokman variety).There was an increase of 12% and 24% in seed germination at third day of treatment with 10ppm and 100pm shilajeet concentrations, respectively. Enhanced growth of root and shoots were recorded on 6th day of germination. Enzymatic analysis of shilajeet treated germinated seeds revealed increase in activity of α-amylase (EC, 3.2.1.1), Starch-phosphorylase (EC 2.4.1.1) and Hexokinase (EC 2.7.1.1), the indicator enzymes of seed germination. However, the 1000ppm treatment exhibited an inhibitory effect on percentage germination, seedling growth and on enzyme activities. Increased enzyme activities were also accompanied by the enhancement of water soluble protein in 10ppm and 100ppm shilajeet treated germinated seeds.

Keywords: Seed germination, Enzymes, Shilajeet, *Triticum astivum*

1. Introduction

Biostimulants are the substances that promote plant growth when applied in micro quantities. They also help plants to withstand harsh environments. In recent years, a growing interest has been observed with natural biostimulating substances. The influence of humic-like substances was studied on hydroponically cultured various crop plants viz. cucumber, maize, pelargonium, and wheat. Treated plants presented a faster development and reached reproductive stage three to five days earlier than control. They also provoked a better efficiency of plant water uptake and improved the mineral nutrition (Morard *et al*., 2011). Humic-like substances increased the cell permeability (Chen *et al*., 1994; Kaya *et al*., 2005). Fulvic acid induced plant growth also has been reported (Rauthan and Schnitzer, 1981; Poapst and Schnitzer, 1971).Very little is known about the mechanism of action of Fulvic acid on plant metabolism. Recent studies prove that humus and fulvic acid significantly affect an increase in seed germination energy, the intensification of seedling growth, the growth in root weight and shoot development (Matysiak *et al*., 2011**;** Katkat *et al*., 2009). Phytohormones like activities of humic substances have been reported in *Fagus sylvaticae* (Pizzeghello *et al*., 2001). Patil (2010) observed biostimulatory effect of potassium humate and deproteinized juice on wheat seed germination and seedling growth.

Shilajeet is an exudate that is pressed out from layers of rock in the most sacred and highest mountains in Nepal, India and other areas. It is composed of humus, Fulvic acid and organic plant material that has been compressed by layers of rock. Humic acid has been isolated and characterized by Agrawal *et al*., (2010). Shilajeet is known to boost the immune system in human beings (Bižanov *et al*., 2012; Ghosal *et al*., 1989). However, there is no report on the effect of Shilajeet on plant metabolism. Therefore, the present investigation was undertaken to see the biostimulatory action of shilajeet on wheat seed germination.

2. Materials and Methods

2.1 Materials

Wheat seeds, Lokman variety obtained from Agriculture University, Gwalior and Shilajeet obtained from Dabur India Ltd. Company, were used for the study.

2.2 Treatment of seeds with Shilajeet

Wheat seeds were surface sterilized with 0.1% $HgCl_2$ solution, washed repeatedly with distilled water and shade

dried. Sterilized seeds were soaked for 8 hours in four different concentrations viz. 0, 10, 100 and 1000ppm aqueous solutions of shilajeet. Seeds were allowed to germinate in sterilized Petri plates lined with moist filter paper (Whatman no.1). Data for percentage germination and enzyme activities were recorded on 3rd, 4th, 5th and 6th days for both treated and untreated wheat seeds. The length of roots and shoot was recorded on 6th day of germination.

2.3 Enzyme Analysis

2.3.1 α-amylase

This enzyme was extracted and assayed by the method of Xiao *et al*., (2006) with slight modifications. Germinated seeds were ground in 0.02% $CaCl_2$ under ice cold conditions and centrifuged at 3000 rpm for 10 minutes in refrigerated centrifuge. The supernatant was used for enzyme assay. The assay mixture contained 1.0 ml starch (2mg), 0.1 ml of enzyme solution and incubated at 37^0C for 20 minutes. After incubation, 1.0 ml Iodine solution was added and optical density was recorded at 700 nm after addition of 5.0 ml of distilled water. The amount of starch was calculated with the help of standard curve. Finally the enzyme activity as µg starch hydrolyzed per minute per mg protein was recorded. The percentage stimulation in enzyme activity was calculated from the observed data.

2.3.2 Starch-phosphorylase

This enzyme was extracted in Citrate-buffer (0.5M, pH 5.9) from germinated seeds and assayed. The assay mixture contained 1.0 ml of citrate-buffer, 0.5 ml of 1% starch, 0.2 ml of Glucose 6-phosphate (0.1M), 0.3 ml of distilled water and 0.5 ml of enzyme solution. After 20 minutes of incubation at 37^0C, the reaction was arrested by the addition of 1.0 ml 20% TCA. The protein precipitated was centrifuged off and the inorganic phosphate in the supernatant was determined by the method of Fiske and Subbaraw (1925). The enzyme activity was expressed as µmole Pi liberated per minute per mg protein. The percentage stimulation was calculated from the observed data.

2.3.3 Hexokinase

The enzyme extract was prepared in ice-cold tris-buffer (0.2M, pH 7.5) containing 0.01M sodium fluoride. The assay mixture contained 0.2 ml tris-buffer, 0.1 ml of 0.1M $MgCl_2$, 0.1 ml of 0.1M ATP, 0.1ml of 0.033M glucose and 1.0 ml of enzyme solution. After 20 minutes of incubation at 37^0C, the reaction was stopped by transferring the tubes in boiling water bath. The amount of glucose left in the reaction was determined by the method of Somogyi (1952). The enzyme activity was expressed as µg Glucose phophorylated per minute per mg protein. The percentage stimulation was calculated from the observed data.

2.3.4 Protein Estimation

The protein content in the supernatant enzyme was determined by the method of Lowry *et al*., (1951) and the percentage change was calculated from the observed data.

2.3.5 Seedling Growth

The length of roots and shoots were recorded on 6th day of germination because it was measurable only on that day.

3. Results and Discussion

3.1. Seed germination

Biostimulatory effects of shilajeet on seed germination is presented in Figure 1. There was an increase up to 12% and 24% of germination on 3rd day with 10 ppm and 100 ppm shilajeet treatment. However, with 1000 ppm, there was a reduction up to 18% in seed germination. On 5th day, there was no effect on germination due to 10ppm and 100ppm shilajeet treatment. However, the treatment of 1000ppm shilajeet reduced germination up to 56%. Humic acid and humic like substances have also been reported to stimulate the seed germination of tomato, pepper, and maize (Kinga *et al*., 2011; Denir *et al*., 2006).

3.2 Enzymes activity

3.2.1 α-amylase

The Biostimulatory effect of shilajeet on the activity of α-amylase is presented in Figure 2.There was an increase in the amylase activity up to 33.16% and 57.08% due to 10ppm and 100ppm shilajeet treatment, respectively on 3rd day of germination. However, the treatment of 1000ppm reduced the activity up to 25.92% on 3rd day. Finally on 6th day, 1000ppm treatment caused reduction of 79.89% in the amylase activity. This clearly indicated a co-relation in the

amylase activity and seed germination. Stimulation in α-amylase during seed germination due to phytohormones and zinc treated maize and bajra seeds have been observed earlier (Shrotri and Jain, 2010).

3.2.2 Starch-phosphorylase

Biostimulatory effect on starch-phosphorylase under the influence of shilajeet treatment is presented in Figure 3. Initially (on 3rd day), there was an increase in the activity up to 55.88% and 87.23% with 10ppm and 100ppm shilajeet treatment, respectively. There was a reduction in the enzyme activity up to 52.94% with 1000ppm shilajeet treatment. It is interesting that there was a gradual decrease in the activity of this enzyme with the age of germination. There was a positive stimulation due to 10ppm and 100ppm shilajeet treatment, however, a negative stimulation due to 1000ppm treatment was observed throughout the investigation.

3.2.3 Hexokinase

The Biostimulatory effect of shilajeet on hexokinase activity is presented in Figure 4.There was an increase in the activity of hexokinase up to 27.75% and 50.04% due to 10ppm and 100ppm of shilajeet treatment, respectively on 3rd day of germination. However, there was a reduction of 16.29% in the Hexokinase activity due to 1000ppm treatment. There was a gradual fall due to 1000ppm in the activity of this enzyme with the age of seed germination.

3.2.4 Water soluble protein

Biostimulatory effect on the amount of water soluble protein during seed germination under the influence of shilajeet treatment is presented in Figure 5.Treatment of shilajeet at the level of 10ppm and 100ppm increased the amount of water soluble protein during seed germination, however, the treatment of 1000ppm exhibited an inhibitory effect on the amount of soluble protein. There was a gradual increase in the soluble protein contents due to 10ppm and 100ppm treatment throughout in the investigation. However, there was a gradual fall in the protein contents with the age of seed germination due to 1000ppm treatment.

3.2.5 Seedling Growth

Biostimulatory effect on root and shoot length due shilajeet treatment is presented in Figure 6. The root length and shoot length increased up to 106% and 121.5% due to 10ppm.Similarly the root and shoot length enhanced 243.9% and 238.2% due to100ppm shilajeet treatment, respectively. The treatment of 1000ppm had inhibitory effect on both root and shoots length up to 48.24% and 73.1% respectively. These results are similar to the observations of Cooper *et al*., (1998) in bent grass due the effect of humic substances on root and shoot growth.

4. Conclusion and Recommendations

These results clearly indicated the biostimulatory effects of shilajeet on seed germination at 10ppm and 100ppm level of treatment. We recommend treating the seeds with shilajeet at 100ppm level prior to sowing in the fields. However, a field trial is required to see the effect of shilajeet on crop yield which is under investigation in our laboratory and fields. Applications of humic substances may help to increase organic food production. Therefore, an agro-biotechnology perspective and programming of the humic like technology needs to be introduced in the world.

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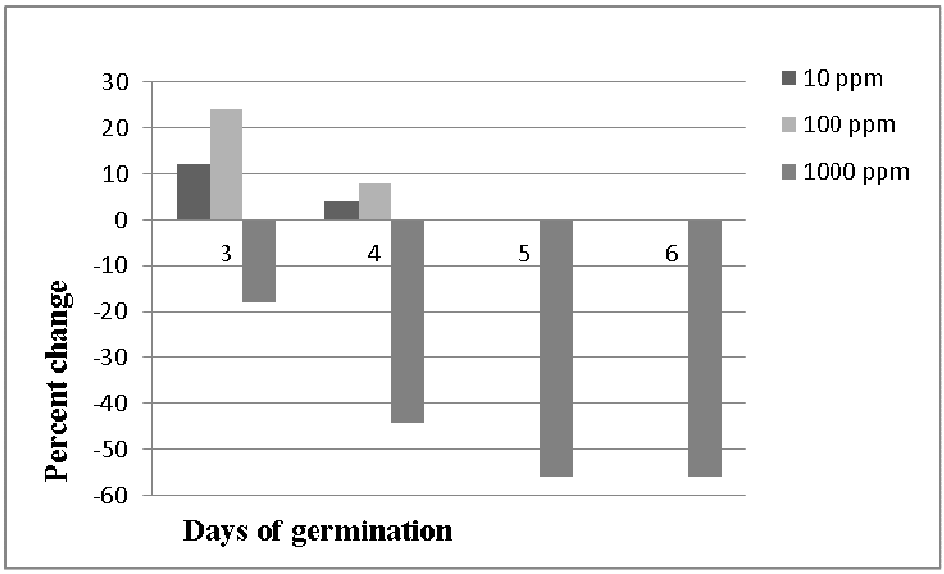

Figure 1. Percent biostimulation in seed germination due to shilajeet treatments

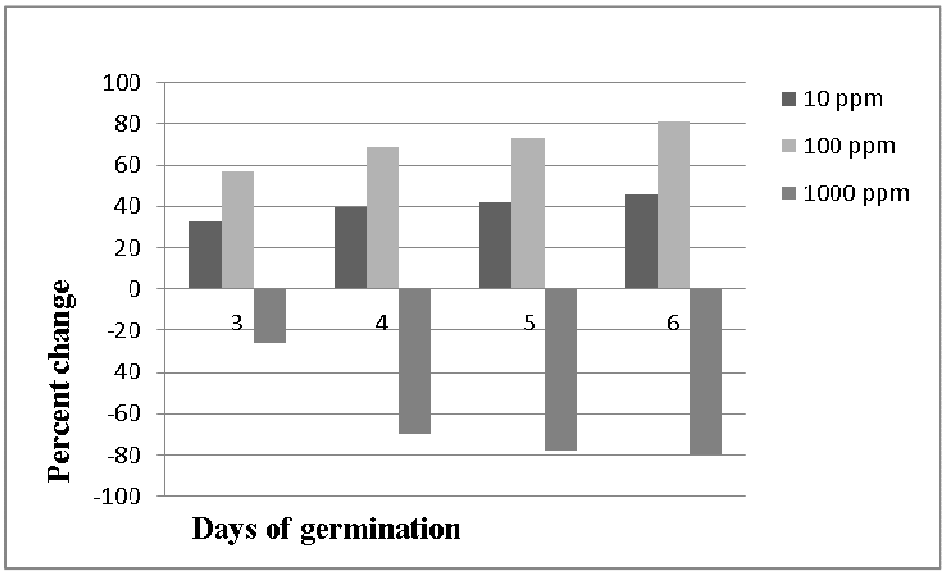

Figure 2. Percent biostimulation in α-amylase activity due to shilajeet treatments

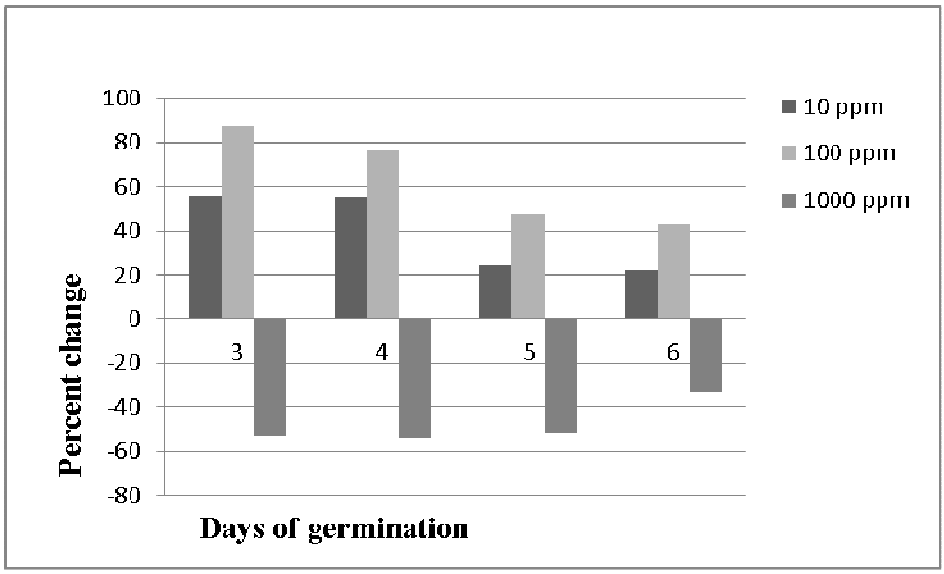

Figure 3. Percent biostimulation in starch-phosphorylase activity due to shilajeet treatments

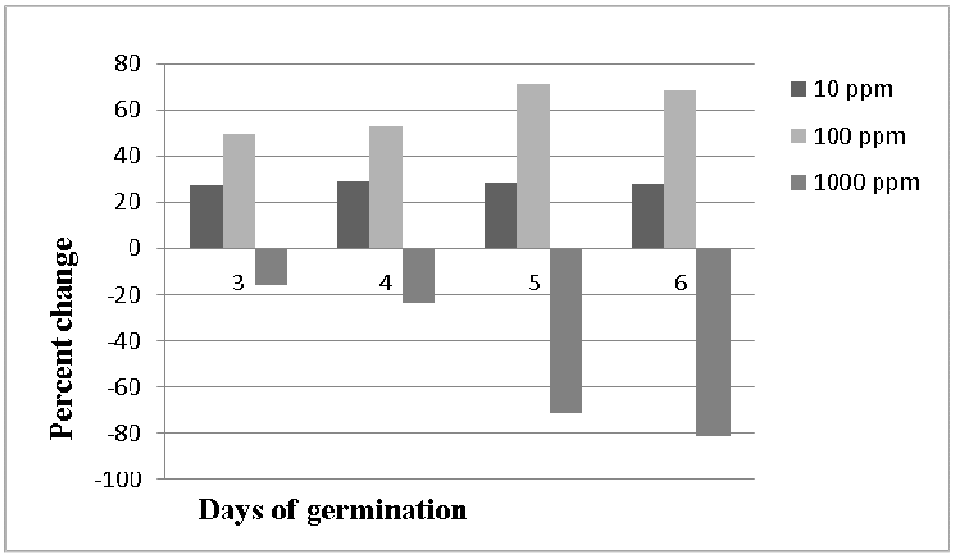

Figure 4. Percent biostimulation in Hexo-kinase activity due to shilajeet treatments

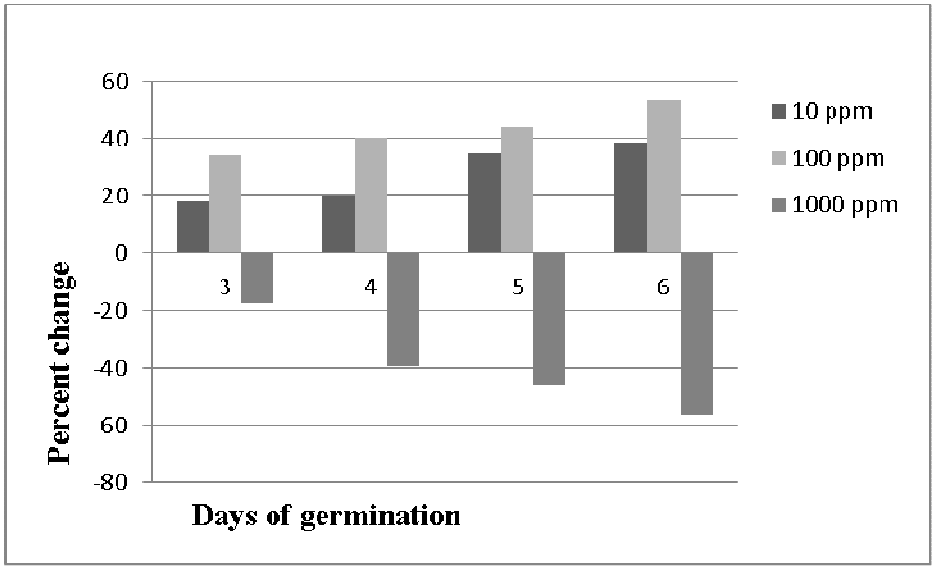

Figure 5. Percent biostimulation in water soluble protein due to shilajeet treatments

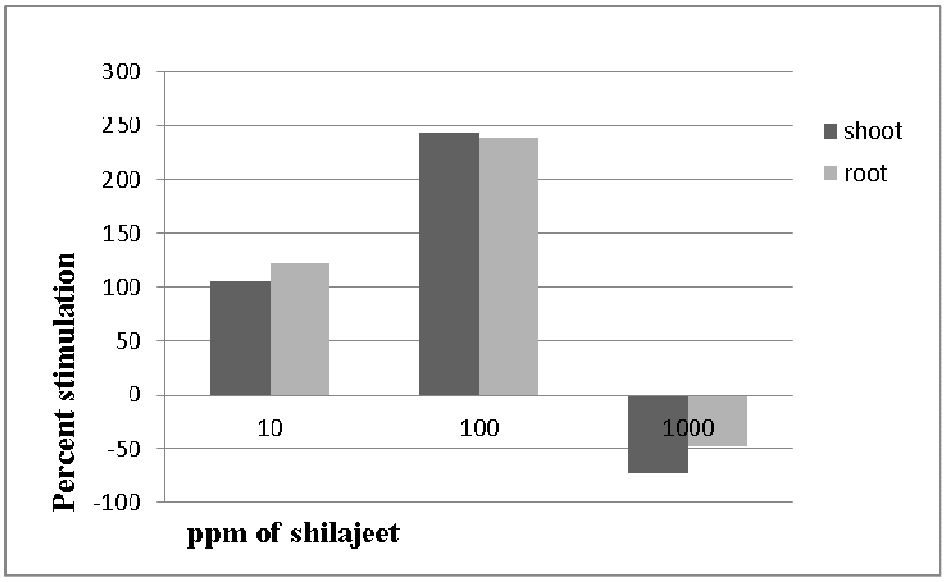

Figure 6. Percent biostimulation in root and shoot length due to shilajeet treatment