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Short Communication

Effect of environmental factors on nitrifying bacteria isolated from the rhizosphere of *Setaria italica* (L.) Beauv

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Nitrification is a biological process in which nitrifying bacteria oxidise ammonia to nitrate. The process is sensitive to environmental factors such as temperature, dissolved oxygen concentration, pH, and available substrate. In the present study, the effects of pH, temperature and neem oil on *Nitrosomonas* and *Nitrobacter* isolated from the rhizosphere of foxtail millet, *Setaria italica*, were examined. The optimum temperature for the growth of both nitrifying bacteria is 30°C. *Nitrobacter* is less tolerant to low temperature than *Nitrosomonas* but the optimum pH for both bacteria is 8.0. Neem oil, when applied at 10 ppm, showed maximum inhibitory effect on growth of both bacteria.

Key words: Nitrifying bacteria, temperature, pH, neem oil, inhibitors, *Setaria italica*.

INTRODUCTION

Nitrogen, a chief and indispensable nutrient for plant growth, often limits crop production. Although it is one of the important nutrients, imperfect water resources, low crop productivity in arid and semi-arid regions and increasing costs of fertilizers have discouraged the extensive use of nitrogenous fertilizers. Hence the need for extensive studies on biological nitrogen fixation. Although increasing nitrogen demand of crops is mainly satisfied by the application of mineral fertilizers, biological nitrogen fixation, a process involving the reduction of atmospheric nitrogen to ammonia by microorganisms, which accounts for nearly 60% of the earth's newly fixed nitrogen (Postgate, 1982), is of great importance in maintaining soil fertility status. *Nitrosomonas* oxidizes ammonia to nitrite, and *Nitrobacter* oxidizes nitrite to nitrate.

The present work endeavor to focus on environmental factors on *Nitrosomonas* and *Nitrobacter* which were isolated from the rhizosphere of Foxtail millet (*Setaria italica* (L.) Beauv).

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MATERIALS AND METHODS

Suspensions of both *Nitrosomonas* and *Nitrobacter* were prepared by adding 2 ml sterile distilled water to the freshly grown slants. From this, 100 µl suspension was inoculated into 50 ml basal medium (pH 8.0) in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (150 rpm) at different temperatures; 20, 30, 40 and 50ºC for 5 days. The pH of the medium was adjusted to different levels; 4, 6, 8, 10 and incubated at 30ºC. In order to determine the effect of neem oil, the medium was amended with 2.5, 5, 10 and 20 ppm of neem oil and the pH of this medium was adjusted to 8 and incubated at 30ºC for 5 days. The same medium was used for both *Nitrobacter* and *Nitrosomonas*, but for the cultivation of *Nitrobacter*, along with the following ingredients, $NaN0_2$ (247.0 mg/ml) was added and pH was adjusted to 8. The composition of the medium is as follows for the culture of both bacteria is (g/l): $(NH_4)SO_4$, 0.235; KH_2PO_4, 0.200; $CaCl_2 2H_2O$, 0.040; $MgSO_4$-$7H_2O$, 0.040; $FeSO_4.7H_2O$, 0.005; $NaEDTA.7H_2O$, 0.005; phenol red, 0.005; and distilled water, 1000 ml.

At desired intervals, aliquots of the growing culture of *Nitrosomonas* and *Nitrobacter* were withdrawn during the course of different experiments. Each sample was monitored for bacterial growth by taking O.D at 550 nm.

RESULTS AND DISCUSSION

The optimum temperature for growth of *Nitrosomonas* and *Nitrobacter* bacteria is 30ºC. Growth rate decreased nearly by 50% at 20ºÇ and 4Õ°C (Figures 1 and 2).

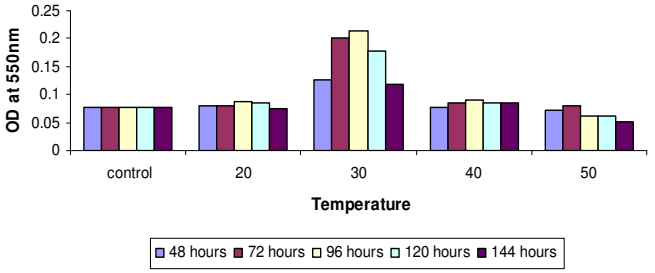

Figure 1. Effect of temperature on growth of *Nitrosomonas*.

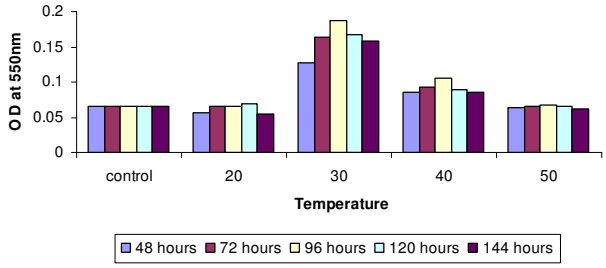

Figure 2. Effect of temperature on growth of *Nitrobacter*.

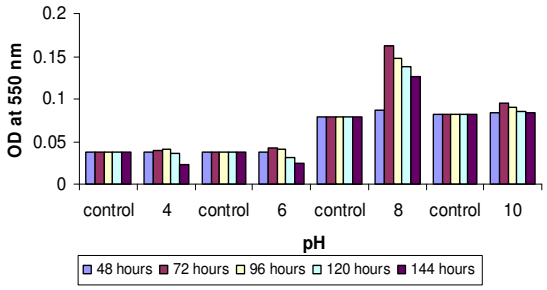

Figure 3. Effect of pH on growth of *Nitrosomonas*.

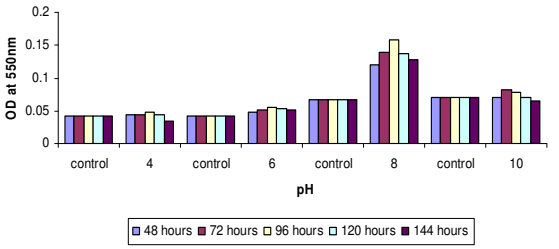

Figure 4. Effect of pH on growth of *Nitrobacter*.

Similarly, both the bacteria exhibited maximum growth at pH 8.0. At pH 4, 6, and 10 the growth rate decreased nearly by 50% (Figures 3 and 4). Amendment with neem oil especially at 2.5, 5.0 and 20 ppm enhanced the growth slightly whereas 10 ppm decreased the growth. However, *Nitrosomonas* and *Nitrobacter* showed

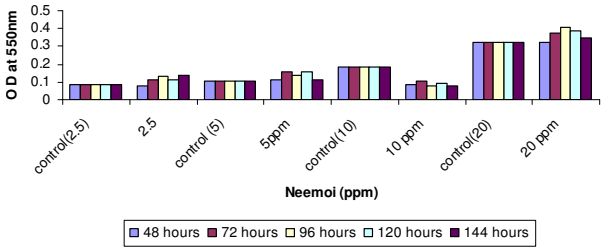

Figure 5. Neem oil effect on growth of Nitrosomonas

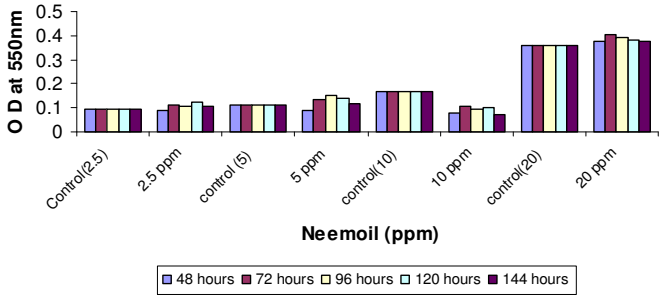

Figure 6. Neem oil effect on growth of *Nitrobacter*.

maximum growth rate at 20 ppm (Figures 5 and 6). Results from our studies confirmed that *Nitrobacter* is less tolerant to low temperature (20ºC) than *Nitrosomonas.* Similar results were also obtained by Alleman (2000). According to Polanco et al. (1994), maximum bacterial activation effect in case of *Nitrosomonas* was observed at 30ºC. Mulvaney (1994) observed that the optimum pH for both nitrifying bacteria was 8.0. The optimum pH is normally between 7.0 and 8.0, but NO_3^- may be formed at a pH of 9.0 or even higher. Das et al. (1999) reported that the growth of nitrifying bacteria was inhibited when neem oil was applied at 10, 15 and 20 ppm. On the contrary our results indicated that neem oil was inhibitory at 10 ppm alone while 2.5, 5 and 20 ppm of neem oil stimulated the growth of the nitrifying bacteria.

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