International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:7, No:10, 2013

Toxic Effect of Sodium Nitrate on Germinating Seeds of *Vigna radiata*

Nilima D. Gajbhiye

Abstract**—**Sodium nitrate has been used industrially in a number of work fields ranging from agriculture to food industry. Sodium nitrate and nitrite are associated with a higher risk of cancer in human beings. In present study, the effect of sodium nitrate on germinating seeds was studied. Two different sets of ungerminated *Vigna radiata* seeds were taken. In one set *Vigna radiata* seeds were soaked in distilled water for 4 hours and they were allowed to germinate in distilled water (Control) and 0.1 to 1% and 10% concentrations of sodium nitrate ($NaNo_3$). In soaked seed set, on 2nd day radical developed in control and 0.1 to 1% concentrations of sodium nitrate. Seeds size was enlarged in 1% and 10% concentrations of sodium nitrate. On 3rd day in 0.1% sodium nitrate length of the radicle was 7.5cm with one leaf let and control sample showed 9cm with one leaflet. On 5th day in 0.1% sodium nitrate length of the radicle was 10 cm with one leaf let and control sample showed 11.5cm with one leaflet. No radicle developed in 1 and 10% $NaNo_3$ concentrations. On 10th day all plants including control were dead. More number of mitotic cells was observed in apical root meristems of control germinating seeds and less mitotic cells were observed in 0.1% $NaNo_3$ germinating seeds. But cells were elongated in 0.9%$NaNo_3$ concentration and particles are deposited in the cells and no mitotic cells were observed. In other sets, dry seeds were allowed to germinate in Distilled water (control) and in 0.1 to 1% and 10% concentrations of sodium nitrate. In dry seed set, on 2nd day radicle developed from control set. In 0.1 to 1% concentrations of sodium nitration seed enlarged in size but but not allowed germination. But in 10% $NaNo_3$ seeds coat colour was changed from dark green to brown. On 3rd day the radicle was developed in 0.1% concentration of $NaNo_3$. No growth of radicle was observed in 0.3 to 10% concentrations of $NaNo_3$ but plumule was observed in control plant. Seed coat color was changed from dark green to brown in color in 1% and 10% $NaNo_3$. On 5th day in control seeds the radicle growth was 11cm and 0.1% $NaNo_3$ concentration was 1.3 cm. On 10th day all plants including control were dead. More number of mitotic cells was observed in apical root meristems of control germinating seeds and less mitotic cells were observed in 0.1% NaNo3 germinating seeds. At higher concentrations of $NaNo_3$ allowed seed germination in soaked seeds but produced radicle decay. In comparison to it, in dry seed set, germination of seeds observed only in 0.1% $NaNo_3$ concentration. The inhibitory effect of $NaNo_3$ on seed germination is due to reduction of water imbibition and mitotic activity.

Keywords**—**Germinating seeds, $NaNo_3$, *Vigna radiate*, mitotic activity.

I. Introduction

THE industrial activity has expanded so much all over the world. Today, it has become matter of major concern in the deterioration of the environment [20]. With the rapid growth of industries in india, pollution of natural water by industrial waste water has increased tremendously [1]. Sodium nitrate is used to make fertilizers. Sodium nitrate acts as the substance that increases the amount of nitrogen contained in the soil. The agriculture field also uses Sodium nitrate to make pesticides. Sodium and potassium nitrates are used as fumigants in canisters, which are placed underground in rodent dens and holes, and then ignited to explode and release gases that kill the rodents [23], [24].

Sodium nitrate is also involved in the manufacture of fireworks as one of its important ingredients. The wastewater industry uses a lot of Sodium nitrate as well. As this polluted water is being used for irrigation to cultivate the crops, Nitrates and nitrites do not volatilize and therefore are likely to remain in water until consumed by plants or other organisms [23], [25].

Sodium nitrate is antimicrobial and it makes Sodium nitrate used largely in the food industry as preservatives [22]. Nitrates have been measured in foods, and have been detected in vegetables and preserved meats [5], [13], [14] and baby foods [3], [8], [10], [18], [21]. Exposure to higher levels of nitrates or nitrites has been associated with increased incidence of cancer in adults, and possible increased incidence of brain tumors, leukemia, and nasopharyngeal (nose and throat) tumors in children in some studies [15], [26]. A number of drugs and plant extracts exhibiting cytotoxic properties are either used or intended to be used for the treatment of various cancers. These drugs interfere with cell-cycle kinetics and inhibit the proliferation of mitotically active cells either by damaging the DNA during S-phase of the cell cycle or by blocking the formation of mitotic spindle in the M phase [6]. The present study is carried out to find effect of sodium nitrate on germinating seeds of *Vigna radiata.*

II. Materials and Methods

A. To Detect the Effect of Sodium Nitate on Seed Germination

Vigna radiata (weighing 49.62 ± 1.50mg) used in this study were obtained from the local market. In one set *Vigna radiata* seeds were soaked in distilled water for 4 hours and seeds were then drained, rinsed twice with distilled H_20. Soaked seeds of *Vigna radiata* were kept in a humidity chamber. The humidity chamber was made by keeping filter paper in petriplates. Different concentrations of sodium nitrate ($NaNo_3$) were made; poured 5ml each day in respective petriplates and distilled water was poured in control sample. Observations were made every day. In another set *Vigna radiata* dry seeds were soaked in distilled water for 4 hours and in different concentrations of sodium nitrate ($NaNo_3$). Soak seeds of *Vigna*

N D Gajbhiye is with the Ramnarain Ruia College, Mumbai, 400019, India (phone: 9221782379; e-mail:drneem@yahoo.com).

radiata were then drained, rinsed twice with distilled H_20 were kept in a humidity chamber for control sample. Different concentrations of sodium nitrate ($NaNo_3$) were made; poured 5ml each day in respective petriplates and distilled water was poured in control sample. Observations were made every day [9], [7].

B. Determination of Mitotic Activity

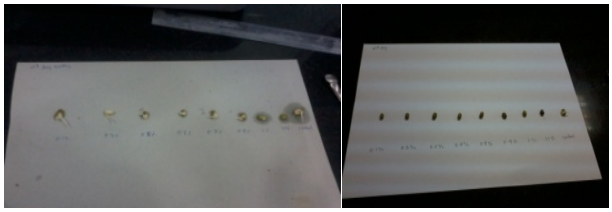
The tips of the radicles (2-3mm) were collected from the germinating seedlings in control sample and different concentrations of sodium nitrate($NaNo_3$) and were placed in 1N HCl for 5 minutes, squashed and stained with 2% aceto-carmine. In each radicle tip the mitotic (meristem cells undergoing mitosis) and total cells were counted in 5-8 fields using light microscope (X100) [2].

III. Result and Discussion

A. The effect of Sodium Nitrate on seed Germination

A B

Fig. 1 A: Soaked seeds of *Vigna radiata* were kept in humidity chambers and different concentrations of $NaNo_3$ were added to respective chambers. B: Dry seed of *Vigna radiat*a soaked in different concentrations of $NaNo_3$



A B

Fig. 2 A: Germination of *Vigna radiata soaked* seeds (Set I) treated with Sodium nitrate concentrations on second day B: Germination of *Vigna radiata* seeds (Set II) treated with Sodium nitrate concentrations on second day

TABLE I
2ND DAY READINGS FOR *VIGNA RADIATA*

$NaNO_3$ (Set I: Soaked seeds)	Germination of seeds	NaNo3 (Set II: Dry seeds)	Germination of seeds
0.1%	1.9cm	0.1%	Size of the seed enlarged but no germination observed
0.3%	1.6cm	0.3%	"
0.5%	1cm	0.5%	"
0.7%	0.9 cm	0.7%	"
0.8%	0.8 cm	0.8%	"
0.9%	0.7cm	0.9%	"
1%	Size of the seed enlarged but no germination observed	1%	"
10%	Size of the seed enlarged but no germination observed but seed coat color changed to dark brown	10%	"
Control	2cm	Control	2cm

TABLE II
3RD DAY READINGS FOR *VIGNA RADIATA*

$NaNO_3$(Set I:Soaked seeds)	Germination of seeds	$NaNo_3$ (Set II:Dry seeds)	Germination of seeds
0.1%	7.5cm	0.1%	0.7cm
0.3%	4cm	0.3%	No germination
0.5%	3cm	0.5%	"
0.7%	1.5cm	0.7%	"
0.8%	0.9cm	0.8%	"
0.9%	0.7cm	0.9%	"
1%	Size of the seed enlarged but no germination observed	1%	"
10%	Size of the seed enlarged but no germination observed but seed coat color changed to dark brown	10%	"
Control	9cm	Control	9cm

TABLE III
5TH DAY READINGS *VIGNA RADIATA*

$NaNO_3$(Set I:Soaked seeds)	Germination of seeds	$NaNo_3$ (Set II:Dry seeds)	Germination of seeds
0.1%	10cm	0.1%	1.5cm
0.3%	5cm	0.3%	No germination
0.5%	4cm	0.5%	"
0.7%	2cm	0.7%	"
0.8%	1.2cm	0.8%	"
0.9%	0.8cm	0.9%	"
1%	Size of the seed enlarged but no germination observed	1%	"
10%	Size of the seed enlarged but no germination observed but seed coat color changed to dark brown	10%	"
Control	11.5cm	Control	11.0cm

A B

Fig. 3 A: Germination of *Vigna radiata soaked* seeds (Set I) treated with sodium nitrate concentrations on Third day B: Germination of *Vigna radiata* seeds (Set II) treated with Sodium nitrate concentrations on Third day

A B

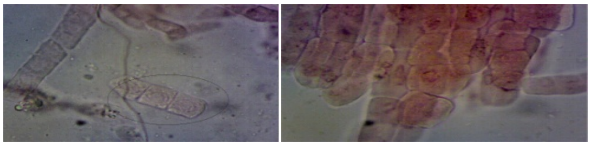
Fig. 4 A: Germination of *Vigna radiata soaked* seeds (Set I) treated with Sodium nitrate concentrations on fifth day B: Germination of *Vigna radiata* seeds (Set II) treated with Sodium nitrate concentrations on Fifth day

A B

Fig. 5: A Germination of *Vigna radiata soaked* seeds (Set I) treated with Sodium nitrate concentrations on Tenth day B: Germination of *Vigna radiata* seeds (Set II) treated with Sodium nitrate concentrations on tenth day

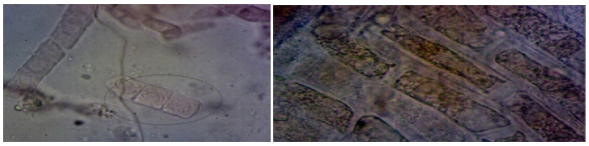
B. The Effect of Sodium Nitrate on Mitotic Cells

Vigna radiata plants grown in 0.1%$NaNO_3$ cells were showed mitotic cells but in 0.9% $NaNO_3$concentration cells were elongated and damaged when compare with control plant.



A B

Fig. 6 A: Mitotic cells were observed in control *Vigna radiata* root tips B: Mitotic cells were observed in 0.1%$NaNo_3$ treated *Vigna radiata* root tips



A B

Fig. 7 A: Mitotic cells were observed in control *Vigna radiata* root tips B: Mitotic cells were not observed , cells in 0.9 %$NaNo_3$ treated *Vigna radiata* root tips

In present study, Fig.1 showed the two sets of seed germination in set I seeds were soaked in distilled water and then kept in humidity chambers different concentrations of sodium nitrate was added and in second set, dry seeds were allowed to germinate in Distilled water (control) and in 0.1 to 1% and 10% concentrations of sodium nitrate. Growth of quiescent embryo in the seed begins as a consequence of triphasic process of water imbibitions in the seeds that finally brings about shedding of seed coat and radicle emergence [2], [4].

Fig. 2 and Table I showed the germination of seeds in soaked seed set I, on 2nd day radical developed in control and 0.1 to 0.9% concentrations of sodium nitrate. Seeds size was enlarged in 1% and 10% concentrations of sodium nitrate. No germination was observed in dry seed set II, on 2nd day radicle developed from control seed. In 0.1 to 1% concentrations of sodium nitration seed enlarged in size but not allowed germination. But in 10% $NaNo_3$ seeds coat color was changed from dark green to brown. The sodium nitrate treatment in set II may have affected water and osmotic potential thus preventing the development of turgor pressure in the seed, which has been considered as one of the key factors for the initiation of radicle growth during seed germination [27].

Fig. 3 and Table II showed increased growth in set I, on 3rd day in 0.1% sodium nitrate length of the radicle was 7.5cm with one leaf let and control sample showed 9cm with one leaflet. In second set II, on 3rd day the radicle was developed in 0.1% concentration of $NaNo_3$. No growth of radicle was observed in 0.3 to 10% concentrations of $NaNo_3$ but plumule was observed in control plant. Seed coat color was changed from dark green to brown in color in 1% and 10% $NaNo_3$. Water imbibitions were allowed in set I seeds. Similar results were observed by [12] but soaking the seedlings in higher concentrations of sodium nitrate in set II allowed growth retardation. The growth retardation with the higher concentrations of sodium nitrate in set II could have also resulted from the inhibition of cell division and radicle protrusion brought about by osmotic stress [4].

Fig. 4 and Table III showed the germination of seeds in soaked seed set I, on 5th day in 0.1% sodium nitrate length of the radicle was 10cm with one leaf let and control sample showed 11.5cm with one leaflet. No radicle developed in 1 and 10% $NaNo_3$ concentrations. In second set II, on 5th day in control a seed the radicle growth was 11cm and 0.1% $NaNo_3$ concentration was 1.3cm. The growth of soaked seed in set I of 0.1%$NaNo_3$ plants were faster as compared to set II 0.1%$NaNo_3$ concentrations. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and water. The similar result was reported [17] the reduction in the amount of water absorption take place with results in to reduction of seed germination due to enhanced effluent salinity.

In Fig. 5 shown *Vigna radiata soaked* seeds (Set I) treated with Sodium nitrate concentrations all plants were dead on tenth day and seed coat colour was changed from green to brown and in (Set II) all plants in control and 0.1% $NaNo_3$ were dead on tenth day and in other concentrations seed colour changed from green to brown.

In present study the growth retardation was observed on 5th day in set I seedlings was associated with in mitotic acivity in the root tips meristematic tissue. Fig. 6 showed more number of mitotic cells was observed in apical root meristems of control germinating seeds and less mitotic cells were observed in 0.1% NaNo3 germinating seeds but cells were elongated in 0.9% $NaNo_3$ concentration and particles are deposited in the cells and no mitotic cells were observed in Fig. 7. The similar results were reported by other workers that the methanol extract of dried latex, the crude dried latex of *C. procera* has earlier been shown to exhibit anti-mitotic activity in the *Allium cepa* model [16]. An association between nitrate exposure and incidence of childhood leukemias was found in one study [11], [14]. Living in areas with high nitrate levels in drinking water during childhood was associated with a higher incidence of testicular cancer [11], [19].

Acknowledgment

The Author would like to thanks for facilities and moral support by Ramnarain Ruia College Mumbai, 400 019, Maharashtra, India.

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Dr. Nilima D. Gajbhiye, Assistant professor, Department of Lifesciecnes, Ramnarain Ruia College, Mumbai, 400019 INDIA.DOB:28/11/1973. Qualifications: M.Sc., D.M.L.T., ph.D.(Lifesciences, University of Mumbai, March, 24th 2003).Member of Mumbai Immunology group 2011, Faculity member of Dept. of Life sciences. Teaching and guiding students of B.Sc. and M.Sc. degree of Mumbai, University for ten years. Has research interest in field of microbiology, environmental science, genetic engineering and immunology. Guided more than 25 short research projects, So for published nine research papers out of which two were read in international conferences in Germany and Mumbai India.