Studies on effect of neem oil and teprosyn on the germination parameters of *Vigna radiate* (L.), wilczek

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**Abstract**

The present investigation was carried out with a biological plant protector, Neem Oil and a chemical yield booster, Terprosyn to observe their effect on the germination parameters of the green gram (*Vigna radiata*). The germination of green gram seeds was inhibited with neem oil treatment but was elevated with Terprosyn. The same was observed in case of Shoot and Root length, fresh and dry weights also. The investigations indicated that the plant protectors like neem oil is to be administered in very low concentrations and for shorter duration since higher concentration and duration is affecting all the germination parameters significantly.

**Keywords:** neem oil, teprosyn, ragi, germination

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**Introduction**

The inorganic and organic forms of heavy metals, insecticides, fungicides, herbicides etc. decrease the germination rate (Cayford and Waldron, 1967). Many reports pertaining to the pesticide toxicity and role of pesticide on seed germination and seedling establishment are available. Kozlowski and Saskan (1970), Dubai and Mall (1975), Mukherjee and Ganguli (1974) worked on the role of pesticides on seed germination and seedling establishments and made appreciable contribution on different crop plants. Padhi (1990) reported significant variations or no germination at all, when rice grains were exposed to MEMC (Methyl Ethyl Mercury Compound), an organic mercurial compound. Hasset et al. (1976) have stated that inhibition of elongation is one of the sensitive parameters to assess the toxicity of heavy metals. Further they stated that, in respect of the growth rate, pesticide was found to be more toxic than mercury, cadmium and zinc. Godbold and Hutterman (1985) observed that the root elongation was severely inhibited by 0.1 to 0.5 ks mode of mercury. The treatment of *Allium cepa* with methoxibeta ethyl mercuric chloride (Venot and Giachhero, 1971) showed no cell division in root and also showed growth inhibition. Treatments of various concentrations of metallic compounds of both inorganic and organic mercury (Mukherji and Ganguli, 1974) on different varieties of rice have shown that at lower concentrations the inhibition of germination was not at all marked rather stimulation was noticed. Padhi (1990) and Siegel et al. (1984) studied the seed germination and early growth of seedlings of 15 species of rice treated with mercury vapor and observed that more than 50% of the plants did not show inhibition of root and shoot length but occasional stimulation was noticed. Sharma (1982) reported that the seeds of different varieties of *Phaseolus aureus* treated with mercuric acetate solution showed inhibition of seedling growth.
Experiments were conducted to study the toxic effect of various mercury compounds such as inorganic monovalent mercurous chloride, divalent mercuric chloride, mercuric acetate and organic compounds of mercury as phenols and mercuric acetate on seed germination, pigment content, root and shoot lengths and fresh and dry weights of barley. It was observed that phenyl mercuric acetate was more toxic, followed by mercuric acetate and mercuric chloride (Mukhiya et al., 1983).

The fresh and dry weight of the seedlings of rice, gram, mustard were also significantly reduced by increasing the concentration of multineem, which might be due to inhibition and/or checking of protein, nucleic acid and carbohydrate synthesis. Padhy et al. (1992), Sahu (1997), Patnaik (1988) on ragi, Sahu (1998) on sesame, Jaya Kumar et al. (1990), Panda (1994) while working on groundnut studied the effect of Eucalyptus leaf leachate globules which contains all electro chemicals like phenolics, terpenoids and flavinoids which are more or less similar in structure and function of multineem. Inhibition of root growth was considered to be an indication of pesticide toxicity and depletion of the shoot growth might be the secondary effect reflected due to root inhibition (Godbold et al., 1984; Godbold and Huttermann, 1985). Acquisition of more amounts of toxic ions by the root from the pesticide contaminated soil might be imparting high toxic effect in root cells. On the other hand the low amount of pesticide probably deposited in shoot might have shown inhibitory action. Very low concentrations of pesticides induce stimulation in growth which was confirmed by Sahu (1998). Stratton and Crake (1979) reported that the low levels of mercuric ions stimulated acetylene reduction and photosynthesis. The review of literature in the preceding paragraphs shows that some amount of work has been done in pesticide toxicity. The present work is one such attempt just to monitor the change in Vigna radiata (L) silczk in response to treatment with neem oil and Teprosyn. But very limited literature is available where work has been done using natural (biological) Plant protectors such as neem oil, neem cake oil. Encouraging report has come out in research involving Plant related protectors in place of chemical protectors. The primary objective of employing plant products is to combat different types of pollution especially water and soil.

Materials and Methods

Vigna radiata (L) Wilczek (Syn. Phaseolus aureus) of the family, Fabaceae popularly known as, ‘green gram or golden gram’ has been chosen as plant material. It is an annual more or less perennial herb with slightly hairy plant. It has slender pointed trifoliate leaves and yellow flowers. Flowers are bisexual, complete, zygomorphic consisting 5 petals and 5 sepals, petals are unequal, polypetalous. Stamens are generally 9 or 10 and carpel is one with unilocular ovary with ovules on marginal placentation. Fruits are generally legumes and produced in clusters on an upright stem. A high yielding variety of Vigna radiata (L) Wilczek was obtained from Regional Research Centre, OUAT, Ratnapur (District Ganjam), Odisha with label PDM52, which was used as the experimental material. Neem oil (biological plant protector) and Teprosyn (a commercial chemical yield booster) were used as the seed treating materials.

Selection of effective concentrations

Pilot experiments indicated that the chemicals exerted both promoting and retarding effects on germination and seedling growth of the plant at different concentrations. Concentrations of 0.04, 0.06, 0.08 ml/L were selected for both neem oil and teprosyn.
**Germination studies**

For germination studies, borosil petridishes (10 cm Dia.) were sterilised in an autoclave. Pure line seeds of *Vigna radiata* (L) Wilczek were selected and surface sterilised with 0.03% (v/v) formalin solution for half an hour and then washed thoroughly with tap water. These surface sterilized seeds were allowed to soak in desired concentrations. Three replications for 3 different concentrations i.e. 0.04, 0.06, 0.08 ml/L and 3 different treatment durations i.e. 2, 3 and 4 hrs were taken up along with 3 controls. Four layers of wet blotting papers were placed in each Petridish and after treatment the seeds were washed thoroughly and kept on blotting papers of petridishes for germination. The blotting papers were made wet at regular interval with distilled water. The petridishes were kept in the dark room at room temperature. The emergence of radical was considered as index of germination. Percentage of germination was recorded at an interval of two days after soaking till 6 days after soaking.

**Morphological studies**

The growth of plant was evaluated by measuring the shoot and root length of seedling on the 7th day. For this all the petridishes of both control and treated sets containing germinated seeds were kept in growth chamber maintained at 30 ± 2°C and provided 10 hr photoperiod/day with white light intensity of 2.0k lux from two fluorescent tube lights. All the petridishes were supplied with equal volume of distilled water as per the experimental design every day during the period of observation to maintain equal moisture 15 cm. Scale was used for the measurement of the shoot and root length. After measuring the length of the shoot and roots the fresh weight of both shoot and roots were measured separately with the help of an electronic balance. The weighed shoot and roots were kept in an oven maintained at 100°C for 24 hr. for determining their dry weights in mgs. The present investigation is studies on the effect of biological plant protector namely neem oil which is generally useful as antiseptic compound and also work as an insecticide and a synthetic chemical yield booster namely teprosyn which normally encourages vigorous seedling growth and maximizes crop yields. The results have been dealt under five categories i.e. germination studies, study of seedling growth, dry and fresh weight studies, changes in biomolecules and changes in enzyme studies.

**Results**

The data collected after experimentation is given in Table 1. Germination of seeds were studied at concentration of 0.04, 0.06, 0.08 ml/L of neem oil. It was found that the percentage of germination decreased over controls. Moreover with increase in the treatment period decrease in the percentage of germination has been observed. Germination of seeds were studied at concentration of 0.04, 0.06, 0.08 ml/L of teprosyn and unlike neem oil it was found that percentage of germination has increased with the increase in treatment period of seeds. There was a linear relationship between the percentage of germination and the concentration of test chemical and time period of seed treatment.
Seedling establishment is considered as one of crucial stage after germination where various morphogenesis activities are accomplished along with changes in physiological and biochemical processes. During this period the seedlings try to become self established by the development of root and shoot system. The growth and development of shoot and root are controlled by various metabolic activities carried out inside the leaves. Hence, changes in shoot and root length of seedlings, their fresh and dry weights and changes in macromolecular contents are considered as important parameters of seedling establishment. The impact of different concentrations of neem oil and teprosyn on seedling growth (at the end of 7 days) of *Vigna radiata* is shown in Table 1. With concentrations of 0.04, 0.06 and 0.08 ml/L and with treatment periods of 2, 3 and 4 hrs the root and shoot length decreased in case of neem oil and increased with increase in the concentration in respect of teprosyn. The root and shoot length decreased in the higher concentrations in neem oil treatment may be due to inhibition of both root and shoot growth. Besides root and shoot lengths fresh and dry weights of the seedling were also taken as the indices of growth. In case of teprosyn treatment, the dry and fresh weight of *Vigna radiata* (L) Wilczek showed increase from concentration 0.04 to 0.08 ml/L. There was a linear relationship between the concentration and seed treatment period also. Increase of treatment periods from 2 to 4 hrs and the concentration from 0.04 to 0.08 ml/L has shown increase in the fresh and dry weights. Unlike teprosyn increase in the concentration of neem oil from 0.04 to 0.08 and the treatment period from 2 to 4 hrs has shown a decline in the fresh and dry weights of the seedlings.

See Table 1 for more details.

### Table 1. Germination parameters in 7 days old *Vigna radiata* seedlings after treatment with Neem oil and Teprosyn

<table>
<thead>
<tr>
<th>Conc. of Neem oil/teprosyn in ml/lit.</th>
<th>Treatment (in hrs)</th>
<th>% of germination</th>
<th>Shoot length (in cm)</th>
<th>Root length (in cm)</th>
<th>Shoot Fresh wt. in mg</th>
<th>Shoot Dry wt. in mg</th>
<th>Root Fresh wt. in mg</th>
<th>Root Dry wt. in mg</th>
</tr>
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<tr>
<td>Control</td>
<td>-</td>
<td>90</td>
<td>13.65</td>
<td>9.21</td>
<td>3500</td>
<td>550</td>
<td>1700</td>
<td>160</td>
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<tr>
<td>Neem oil 0.04 ml/L</td>
<td>02h</td>
<td>70</td>
<td>12.18</td>
<td>7.92</td>
<td>2880</td>
<td>460</td>
<td>1610</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>03h</td>
<td>68</td>
<td>11.82</td>
<td>7.06</td>
<td>2760</td>
<td>380</td>
<td>1400</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>04h</td>
<td>64</td>
<td>10.68</td>
<td>6.92</td>
<td>2680</td>
<td>345</td>
<td>690</td>
<td>75</td>
</tr>
<tr>
<td>0.06 ml/L</td>
<td>02h</td>
<td>64</td>
<td>11.30</td>
<td>7.25</td>
<td>2820</td>
<td>440</td>
<td>1600</td>
<td>125</td>
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<tr>
<td></td>
<td>03h</td>
<td>60</td>
<td>10.56</td>
<td>6.22</td>
<td>2710</td>
<td>362</td>
<td>1250</td>
<td>105</td>
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<tr>
<td></td>
<td>04h</td>
<td>56</td>
<td>9.72</td>
<td>6.25</td>
<td>2200</td>
<td>280</td>
<td>650</td>
<td>60</td>
</tr>
<tr>
<td>0.08 ml/L</td>
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<td>60</td>
<td>8.75</td>
<td>6.58</td>
<td>2790</td>
<td>410</td>
<td>1550</td>
<td>135</td>
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<tr>
<td></td>
<td>03h</td>
<td>56</td>
<td>8.17</td>
<td>5.36</td>
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<td>350</td>
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<td>110</td>
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<td>04h</td>
<td>50</td>
<td>7.41</td>
<td>5.58</td>
<td>1080</td>
<td>240</td>
<td>600</td>
<td>40</td>
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<td>Teprosyn 0.04 ml/L</td>
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<td>15.74</td>
<td>10.95</td>
<td>3605</td>
<td>567</td>
<td>1800</td>
<td>280</td>
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<td></td>
<td>03h</td>
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<td>16.87</td>
<td>12.27</td>
<td>3750</td>
<td>610</td>
<td>2080</td>
<td>310</td>
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<td>04h</td>
<td>95</td>
<td>18.84</td>
<td>14.06</td>
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<td>02h</td>
<td>94</td>
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<td>18.42</td>
<td>12.82</td>
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<td>613</td>
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<td>20.66</td>
<td>14.34</td>
<td>4000</td>
<td>750</td>
<td>2300</td>
<td>350</td>
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<td>0.08 ml/L</td>
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<td>12.07</td>
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<td>630</td>
<td>2110</td>
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<td>15.01</td>
<td>4100</td>
<td>760</td>
<td>2310</td>
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</table>

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Discussion

Germination is sum of all the physiological processes occurring inside the seed that starts with the imbibition of water and end with the protrusion of embryonic root in dicots and coleorhiza in monocots. The seeds of *Vigna radiata* (L.) Wilczek PD52 showed 100% germination during September-December. There was much of variation in the percentage of germination during experimental schedule. In general, decrease in germination has been one of the important manifestations of pesticide toxicity as reported in many plants (Baiku and Dianocu, 1972; Sorteberg, 1974; Lipsey, 1978; Brown, 1978; Butler, 1978; Siegal et al., 1984; Padhi, 1990). However, the growth regulator Teprosyn shows increase in the germination. Inhibition of root and shoot growth is due to the effect of pesticides was reported by Lipsey (1978), Sorteberg (1974), Weaver (1984), Hasset et al. (1976). Stimulation of root length in rice plant was observed by Siegel et al. (1984). They studied the seed germination and seedling growth of 15 different species of rice plants treated with organic mercurial compound and observed that more than 50% plant did not show inhibition of root and shoot length but occasional stimulation was noticed.

It has been a well established fact that high rate of germination with rapid vigor of the seedling is an important index of the yield potential of crops. Teprosyn is a synthetic chemical which is used as yield booster. It encourages vigorous seedling growth. It maximizes crop yields by nourishing crops at critical early stages. Its rate of application for 1 kg of seeds is 12 ml for cotton, 8 ml for maize, 15 ml for chillies and 10-15 ml for vegetables. It ensures germination even under unfavorable conditions such as drought or extremely wet or cold weather. The concentrations used in the present investigation are 0.04, 0.06 and 0.08 ml/L. In other concentrations seedlings were found to have stunted growth. The duration of treatment period is 2, 3 and 4 hrs. Use of Teprosyn not only secures rapid germination but also develops strong and healthy shoots.

Similar concentrations were used for treatment with neem oil also but here the root, shoot length, dry and fresh weight and all chemical contents decreased with increase of concentration as well as duration. Decrease in shoot and root length with increase in concentration of neem oil suggest toxicity of ions (Panigrahi and Misra, 1978). It has also been shown that retarding effect of salt on seedling establishment is directly proportional to osmotic pressure it exerts. The neem oil increases the osmotic pressure of the medium, thereby reducing the availability of water needed for the cotyledon and axis which finally leads to the decreasing the seedling establishment. Retardation in Shoot and root growth was probably due to hormonal damage or due to the irregular hormonal transportation. The decrease in dry weight may be correlated with the loss owing to respiratory activities in the endosperm and developing axis. The moisture content referred to the metabolic changes had no such remarkable changes. In higher concentration it decreases due to hydrolysis of reserve components in the seed (Nouri et al., 1970). It was found that in lower concentration seedlings showed retardation and in higher concentration the seedlings decay. Retardation caused by mitotic failure and restriction in elongation, differentiation and maturation are due to water deficit. Growth retards in the seedling at higher concentration and their death may be linked with inhibition of metabolic reaction and damage caused to membrane. This has been due to an excessive loss of soluble metabolites from the seed to the outer medium. As the root is the primary absorbing organ, in a
toxic solution phase, it is likely to manifest the toxic symptoms which are more remarkable. Many different pesticides are continuously used in agriculture for killing pests, insects, fungus, bacteria, rodents, weeds etc. under different brand names and these pesticides are sprayed in the agriculture field as mass spray in a particular dosage as recommended by the manufacturing companies. This dose/concentration is selected after very careful scrutiny in such a way that it will not kill the crop plants, but can kill the pest, insect, pathogen, fungus or bacteria. It is not true that these pesticides have no effect on these crop plants. These pesticides show number of changes and damages (such as physiological changes and bimolecular changes in crop plants). The present investigation is aimed at the effect of biological plant protector such as neem oil which is an insecticide and a chemical yield booster named teprosyn which is a synthetic chemical both taken for the study of germination and seedling metabolism of the seeds of Vigna radiata (L) Wilczek.

Conclusion

The concentrations chosen for both teprosyn and neem oil are 0.04, 0.06, 0.08 ml/L and the treatment periods for each concentration are 2, 3 and 4 hrs. It was observed that the length, fresh and dry weights of root and shoot in teprosyn treated lines has shown an increase with increase in concentration and treatment period. Where as in the neem oil treatment the increase of concentration and duration of treatment decreased the root and shoots length, dry and fresh weight. The higher treatment period of seeds even caused toxicity to the seedlings.

Acknowledgements

Authors are thankful to Principal and Head of the Department of Botany of S.K.C.G (A) College, Paralakhemundi and Principal and Head of the Department of Botany and Biotechnology of Khallikote (A) College, Berhampur for providing research facilities and for encouraging research activities.

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