CHAPTER 11

EFFECT OF INDOLE-3-ACETIC ACID ON GERMINATION AND SEEDLING GROWTH OF VIGNA UNGUICULATA (L) WALP AND PHASEOLUS VULGARIS SPECIES.

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ABSTRACT

The effect of Indole-3- acetic acid (IAA) on germination and seedling growth of two bean species naely Vigna unguiculata L. Walp and Phaseolus vulgaris was carried out. The germination percentage (FGP) was highest in 0ppm (80%) of V. unguiculata but highest in 100ppm (43.33%) of P. vulgaris. Germination rate index (GRI) was maximum in 0ppm (63.33%/day) followed by 100ppm (35.83%/day) while the minimum value was in 200ppm (6.67%/day) in V. unguiculata. For P. vulgaris, the maximum was 26.67%/day in 50ppm while the minimum was 12.50%/day in 0ppm. Germination first occurred 1 day after planting in 50ppm, 100ppm, 150ppm and 0ppm for V. unguiculata but 2 days after planting in 200ppm. For P. vulgaris, germination was first recorded in 50ppm, 150ppm, 200ppm and 0ppm but 2 days after planting in 100ppm. Germination was last reported 2 - 4 days after planting in both species of beans. The time spread of germination ranged between 0 – 3 days in V. unguiculata and P. vulgaris. The radial length was 16.12mm in 0ppm, 9.86mm in 50ppm and minimum 6.20mm in 200ppm for V. unguiculata while P. vulgaris 7.33mm in 100ppm, 7mm in 0ppm and least 6.11mm in 200ppm. In V. unguiculata, the low concentration of IAA enhances germination while high concentration tends to inhibit germination. In P. vulgaris, pre-soaking of the seed have no effect on the germination as less than 50% of the seeds germinated. The radicle length showed that P. vulgaris. Further studies should be done using other growth hormone on P. vulgaris to improve its germination and seedling growth.

***Key Words**: Indole-3-acetic acid, germination, seedling growth, Vigna uncuiculata L., Phaseolus vulgaris.*

INTRODUCTION

The growing population demands an equal growth in the rate of production of food crops to meet the ever increasing demand for food. In developing countries, the only way to meet this growing demand is by increasing the productivity of food crops, as there is no or less opportunity for opening up new arable lands for agriculture due to population density and urbanization. Chemical methods have been used to increase the productivity of food crops by promoting plant growth and controlling plant pathogens. High inputs of agro-chemicals cause negative environmental effects such as pollution, death of non-target micro-organisms, residue accumulation in soil and finally affect soil fertility. All these negative effects of synthetic fertilizers lead to the search of more sustainable and eco friendly agricultural practices. Soil organic matter and beneficial soil microbes are of growing importance as key factors in maintaining soil quality and crop production. Indole acetic acid (IAA) and gibberlic acid (GA3) can manipulate a variety of growth and developmental phenomena in various crops. IAA has been found to increase the plant height, number of leaves per plant with consequent enhancement in seed yield in Cotton (Kapgate et al., 1989). It also increases the flowering, fruit set, and the total dry matter of crops (Gurudev and Saxena, 1991). Likewise, GA3 stimulated stem elongation (Harrington et al., 1996), and enhance total yield (Deotale et al., 1998).

Seeds are best means of regeneration and this could be achieved through several pretreatments such as acids, distilled water and hormonal preparations at different low concentrations to induce the seed germination and seedling growth/development. Plant hormones are chemicals that regulate plant growth, which, in the United Kingdom, are referred to as 'plant growth substances' (Srivastava, 2002). Plant hormones are not nutrients, rather they are chemicals that in small amounts promote and influence the growth, and development of plant cells and tissues (Srivastava, 2002). Examples of hormones used include Indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA) and gibberellic acid (Ga3).

Germination as the first stage of plant development is one of the critical stages in the life cycle of plants and is a key process in the emergence of seedling (De-Villiers et al., 1994). One way to increase germination and seed emergence, especially under stress conditions, is the use of priming. Seed priming is practicing water treatments (sometimes other materials are associated with water) on the seeds before planting to enhance germination,

initial establishment, early maturity, and improvement of plant growth, which consequently results in the acceleration and uniformity of germination and increase of crop quality and quantity (Toselli and Casenave, 2007). Seed priming techniques are used to improve germination, to reduce the time between sowing and emergence, and for uniform emergence in the field, especially under adverse environments (Gupta et al., 2008).

Several studies have been conducted on physiological and biochemical effects of priming on various species of legumes such as cowpea, pea and lentil which have shown that seed priming can improve germination (Ghasemi, 2008). In environments that are faced with adverse conditions, the use of seed priming can significantly reduce product loss (Abolrahmani et al., 2010).

Plant Growth Regulators

Plant growth regulators are important factors affecting plant growth and development. Salicyclic acid (SA) is a phenolic compound that is produced by root cells. This substance exits in plants in small amount (mg g-1 fresh weight or even less). Salicyclic acid plays a central role in the regulation of various physiological processes during plant growth and development such as ion adsorption, photosynthesis, and germination, depending on the concentration used, species, growth period and environmental conditions (Iqbal et al., 2006). Naphthalene acetic acid (NAA) is another plant growth regulator and an important synthetic auxin used in plants.

LITERATURE REVIEW

Cowpea (Vigna unguiculata (L) Walp) (Leguminosae: Papilionoidae) represent the main food legume and a versatile crop in tropical Africa. It is drought tolerant and could produce better growth in warm climates. It is most popular in the semi arid regions of the tropics where other food legumes are available (Singh and Sharma, 1996). The crop has been described as the major source of dietary protein in tropical and subtropical regions of the world especially where animal protein consumptions are low (Opareke et al., 1998). Efforts made to maximize yield, is largely hampered by adverse effect of a biotic stress such as salinity and drought. These effects cause a huge loss due to law yield and failure of the crop to establish in some cases. Alternative approach towards efficient and cost effective means of production of cowpea in the tropical Savannah is very desirable.

Pre-sowing hardening seed treatment is an easy, low cost and low risk

technique and also an alternative approach recently used to overcome the effect of abiotic stresses in agricultural production. It is found to be efficient in improving seed emergence and growth of crops. Pre-sowing hardening treatment is a repeated soaking and control seed hydration in solution containing organic or inorganic solute (Pill and Necker, 2001), followed by redrying that allows pre-germinative metabolic activities but prevent radical emergence (Basra et al., 2003). The hardening treatment proved to be better for vigour enhancement than the traditional soaking (Basra, et al., 2005). It was reported clearly that the hardening treatment enhance seeds vigour by protecting structure of the plasma membrane against injury during stress (JunMin et al., 2000). It is a well establish fact that, pre-soaking seeds with optimal concentration of phytohormones enhance their germination, growth and yield of some crop species under condition of environmental stress by increasing nutrient reserves through increased physiological activities and root proliferation.

Previous studies have also shown that presowing seed treatment in various concentration of Indole acetic acid (Gulnaz et al., 1999), Gibberellic acid (Radi et al., 2001 and Anguish et al., 2001) and Ascorbic acid (Roy and Srivastava, 2001; Alhakimi and Hamada, 2001) may promote or inhibit seedling growth. However little is emphasized on how plant growth hormones could affect cowpea seed germination and seedlings growth. The main objective was to assess the physiological effect of Indole3acetic acid (IAA), Gibberallic acid (GA3) and Ascorbic acid (AA) on germination and seedling growth of cowpea. Al-Desuqey, *et al*.,(2007) reported that IAA, gibberellic acid or kinetin at different concentrations stimulated the growth vigor(root length, root fresh and dry weight, shoot length, shoot fresh and dry weights and leaf area production)of cowpea throughout the growth periods. Also, Sinsiri and Laohasiriwong (2007) working on cowpea using different rates of IAA and found that root length, and number of both roots and root hairs were highly affected by IAA treatments and the best IAA level was found with level 3 (500 mg/litre). Meanwhile, foliar application of indole acetic acid (IAA) at three concentrations (12.5, 25 and 50 ppm) induced increments of the plant height, fresh and dry weights, number of branches and number of leaves per plant as well as yield components (pods per plant, seeds per pod, weight of pod, weight of seeds per plant and weight of seeds/feddan) (El-Bassiouny and Shukry, 2001). Several investigators also reported an increase in yield of different plants by application of auxins (Rao and Narayanan, 1997).

Germination as the first stage of plant development is one of the critical stages in the life cycle of plants and is a key process in the emergence of seedling. One way to increase germination and seed emergence, especially under stress conditions, is the use of priming. Seed priming is practicing water treatments (sometimes other materials are associated with water) on the seeds before planting to enhance germination, initial establishment, early maturity, and improvement of plant growth, which consequently results in the acceleration and uniformity of germination and increase of crop quality and quantity (Toselli and Casenave, 2007). Seed priming techniques are used to improve germination, to reduce the time between sowing and emergence, and for uniform emergence in the field, especially under adverse environments (Gupta et al., 2008).

Several studies have been conducted on physiological and biochemical effects of priming on various species of legumes such as cowpea, pea and lentil which have shown that seed priming can improve germination (Ghasemi, 2008). In environments that are faced with adverse conditions, the use of seed priming can significantly reduce product loss (Abolrahmani et al., 2010). Plant growth regulators are important factors affecting plant growth and development. Salicylic acid (SA) is a phenolic compound that is produced in plants by root cells. This substance exists in plants in small amounts (mg g-1 fresh weight or even less). Salicylic acid plays a central role in the regulation of various physiological processes during plant growth and development such as ion adsorption, photosynthesis, and germination, depending on the concentration used, species, growth period and environmental conditions (Iqbal et al., 2006). Naphthalene acetic acid (NAA) is another plant growth regulator and an important synthetic auxin used in plants.

The low fertility status of most tropical soils had hindered cowpea production, as cowpea is a high nutrient demanding crop and generally, the crop fail to produce good yield in plots without adequate nutrients (Adediran and Banjoke, 2003) and inorganic fertilizers play a key role in plant growth and yield (Stefano et al. 2004). The nutrient from inorganic fertilizers will lead to improve cell activities, enhanced cell multiplication and enlargement and luxuriant growth (Fashina et al. 2002) which result in high dry matter production (Obi et al. 2005). Some factors, which may also contribute to low yield of cowpea, are low plant population on farmer's plots, use of unimproved seeds for planting, weed not controlled especially all critical

periods of competition, pests and diseases. However, the use of plant growth regulators can help. Plant growth regulators are known to affect germination and growth of plant. This study seek therefore to determine the effect of different concentrations Auxin / Indole-3-acetic acid on the seed germination and seedling growth of cowpea for subsequent recommendation (in agroforestry and to enhance large scale propagation of the species for plantation establishment).

The application of growth hormone increase the seed germination percentage by increase of the amino acid content in embryo and they cause release of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination. The overall development of plant is regulated by the growth hormones, nutrient and environmental factors. They also vary in their germination requirement (Chauhan, 2010). It is not known in which concentrations this hormone will cause a response in the cell. This investigation with growth hormone will help in determining which of the hormonal concentration is suitable for seed germination and proper seedling growth. This analysis is considered necessary since the beneficial effect of presoaking treatment of seeds with plant growth regulator and other substances have been reported in the present literature repeatedly. The aim and objectives of the study: (i) to evaluate the effect of Indole-3-acetic acid on the germination rate of cowpea (ii) to determine the highest germination rate based on concentration of Indole-3-acetic acid (iii) to determine the effect of Indole-3-acetic acid on seedling growth of cowpea.

MATERIALS AND METHODS

Study Area

The research was carried out in the Department of Plant Science and Biotechnology Laboratory, Imo State University, Owerri, Imo State, Nigeria. it is situated between latitude $4^{0}45^{1}5.5037^{0}$N and $7^{0}15^{1}6.453^{0}$N and longitude $6^{0}50^{1}7.0438^{0}$E and $7^{0}25^{1}8.1330^{0}$E. The area has an annual rainfall of about 2500mm, temperature range from 270C to 300C with a relative humidity of 75%.

Seed collection

Certified seeds of Cowpea were collected from Imo Agricultural Development Programme Centre (ADP), Ministry of Agriculture, Owerri. Two varieties of cowpea seeds were obtained namely: *Vigna unguiculata* (L) Walp (commonly known as Black-eyed peas) which is white, rough in texture and

Phaseolus vulgaris (common beans), brown in colour and also rough in texture. These seeds were sterilized after weighing using 75% ethanol in 250ml conical flask.

Preparation of the IAA solution

1g of IAA was weighed and put in beaker. The IAA was dissolved with 5ml of ethanol and 1000ml of distilled water added to it. 5ml, 10ml, 15ml and 20ml of the IAA solution was measured and dissolved in 100ml of distilled water each to form 50ppm, 100ppm, 150ppm and 200ppm respectively. 5ml of ethanol was added to the distilled water to get 0ppm which stands as the control.

Pre-sowing hardening treatments

Different concentrations of the growth substance prepared in the laboratory were transferred from the reagent bottles into 50mls conical flasks which were clearly labeled according to the concentration of the growth substance to be used in the soaking treatment. Some quantities, 50ppm, 100ppm, 150ppm and 200ppm each of Indole acetic acid (IAA) was used for soaking the seeds separately. The control was distilled water treatment which was presoaked before sowing. The seeds were soaked in the various concentrations of the growth substance and the distilled water for a period of 12 hours after which they were drained using cotton wool and allowed to dry on filter papers for 24 hours before sowing. Distilled water was also used for soaking and also serves as control (Darra et al., 2017) so that the effect of seed pretreatment on plant growth should not be affected by the differences in seed development along with untreated seeds for comparing the effect of various pre-treatments.

Planting of seeds

After drying, the seeds were sown on moist filter papers in 9cm well labeled Petri dishes. Into each Petri dish, 10 seeds were sown for each concentration and this was replicated 3 times making a total of 30 samples (Petri dishes). The Petri dishes was covered and left by the window side of the laboratory. The seeds were inspected at interval and moistened regularly with water. Observation was made daily for 7 days period during which any seeds germinated was recorded. This was maintained for a period of 3 weeks after which growth of seedlings (shoot length and radical length) were measured using a thread and meter rule. These procedures were replicated three times at intervals to facilitate computation of the recorded data.

The experimental design

The experimental design was laid out in a completely randomized design. All the means and percentages were arranged. Data obtained were subjected to bar-chart using Genstat statistical software (1995 version).

Germination parameters measured

Final Germination Percentage (FGP): This was calculated on the basis of number of seeds germinated and expressed as in percentage.

$$FGP = \frac{\text{Final number of seeds germinated}}{\text{Total number of seeds planted}} \times 100$$

Mean Germination Time (MGT): This represents the mean time a seed requires to initiate and end germination.

$$MGT = \frac{\in F.x}{\in F}$$

Where F = seeds germinated, x = day of germination.

Germination Rate Index (GRI): It gives an indication of the percentage of seeds germinating per day of the test run period.

$$GRI = \frac{G1}{1} + \frac{G2}{2} + \ldots\ldots\ldots \frac{Gx}{x}$$

Where G1 = Germination percentage in day 1 x 100, 1, 2, x = No of days for germination.

First Day of Germination (FDG): The day the first germination event occurs.

Last Day of Germination (LDG): The day the last germination event occurs.

Time Spread of Germination (TSG): This is the time elapsing between the FDG and LDG

TSG = LDG – FDG

Seedling Vigour

Radicle length (cm): The measurement of the radicle using meter rule.

RESULTS

Results of germination of two varieties of beans seeds soaked in different concentrations of IAA was summarized in Tables 4.1 - 4.4 and Figure 4.1 – 4.4. For *Phaseolus vulgaris*, germination parameters showed that Mean Germination Time (MGT) varied from 1.88 days in 50ppm, 1.95 day in 100ppm, 2.50days in 150ppm, to 2 days in 200ppm but 1.54 day in 0ppm (Fig. 4.1). Germination Rate Index (GRT) was recorded to be 31.67%/days in 50ppm, 35.83%/days in 100ppm, 20.84%/days in 150ppm, 6.67%/days in 200ppm while 63.33%/day in 0ppm (Table 4.1). First Day of Germination (FDG) was recorded as 1 day in 50ppm, 100ppm, 150ppm and 0ppm respectively while 2 days in 200ppm (Fig. 4.1). Last Day of Germination (LDG) was 2 days in 50ppm and 200ppm but 4 days in 100ppm, 150ppm and 0ppm respectively (Table 4.1). The Time Spread of Germination indicated 1 day for 50ppm, 0 day for 200ppm but 3 days for 100ppm, 150ppm and 0ppm (Fig. 4.1). Final Germination Percentage varied from 56.67% in 50, to 63.33% in 100ppm, t0 40% in 150ppm, to 13.33% in 200ppm and 80% in 0ppm (Table 4.1). The radical length ranged from 9.86mm in 50ppm, to 7.91mm in 100ppm, to 8mm in 150ppm, to 6.20mm in 200ppm while 16.12mm in 0ppm (Fig. 4.2).

For *Vigna unguiculata*, the Mean Germination Time (MGT) was 1.55 day for 50ppm, 1.56 day for 150ppm while 2 days for 100ppm, 200ppm and 0ppm (Fig. 4.3). The Germination Rate Index (GRT) was 26.67%/day for 50ppm, 21.67%/day for 100ppm, 21.66%/day for 150ppm, 14.17%/day for 200ppm while 12.50%/day for 0ppm (Table 4.2). The First Day of Germination (FDG) was 2 days for 100ppm, 1 day for 50ppm, 150ppm, 200ppm and 0ppm respectively (Fig. 4.3). The Last Day of Germination (LDG) was 2 days for 50ppm, 100ppm and 150ppm but 4 days for 200ppm and 0ppm (Fig.4.3). Time Spread of Germination (TSG) was recorded as 0 day in 100ppm, 1 day in 50ppm and 150ppm, while 3 days in 200ppm and 0ppm (Table 4.2). The Final Germination Percentage was 36.67% in 50ppm, 43.33% in 100ppm, 30.00% in 150ppm, 23.33% in 200ppm while 20.00% in 0ppm (Fig. 4.3). The length of radical was 6.67mm in 50ppm, 7.33mm in 100ppm, 5.89mm in 150ppm, 6.11mm in 200ppm and 7.00mm in 0ppm.

TABLE 4.1: Germination Parameters of *Phaseolus vulgaris* seeds soaked in Different Concentration of IAA

Parameters	50ppm	100ppm	150ppm	200ppm	0ppm
MGT (day)	1.88	1.95	2.50	2.00	1.54
GRT(%/day)	31.67	35.83	20.84	6.67	63.33
FDG (day)	1	1	1	2	1
LDG (day)	2	4	4	2	4
TSG (day)	1	3	3	0	3
FGP (%)	56.67	63.33	40	13.33	80

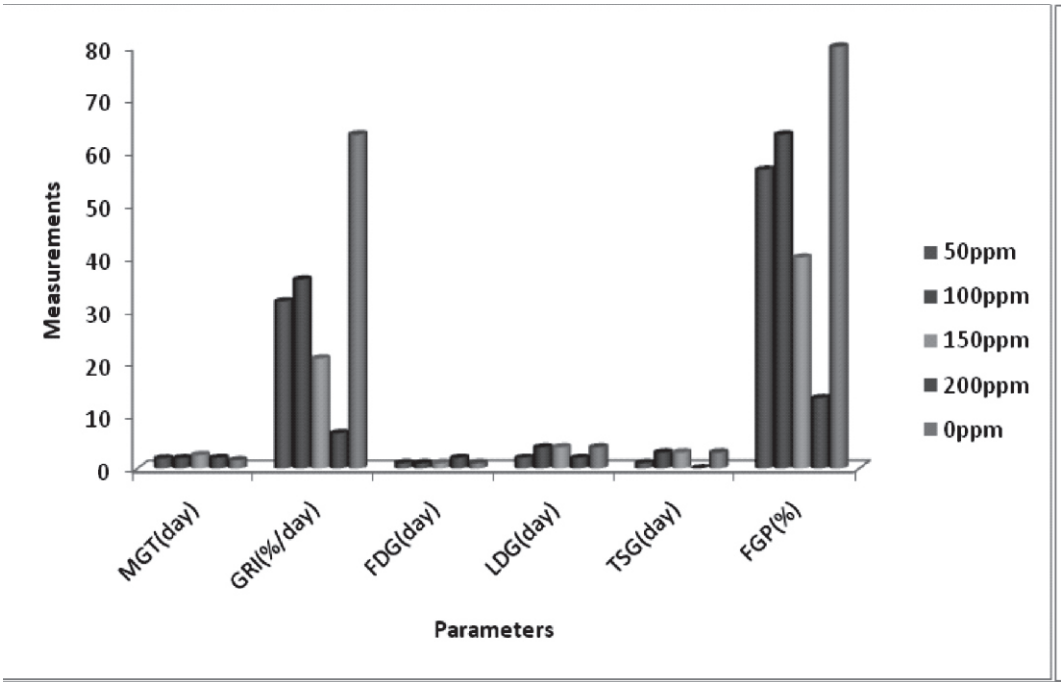

Fig. 4.1: Germination parameters of Phaseolus vulgaris seeds
MGT = Mean germination time, GRT = Germination rate time, FDG = Final day of germination, LDG = Last day of germination, TSG = Time spread of germination, FGP = Final germination percentage.

TABLE 4.2: Radicle Length of Phaseolus vulgaris

Parameters	50ppm	100ppm	150ppm	200ppm	0ppm
Radicle length(mm)	9.86	7.91	8.00	6.20	16.12

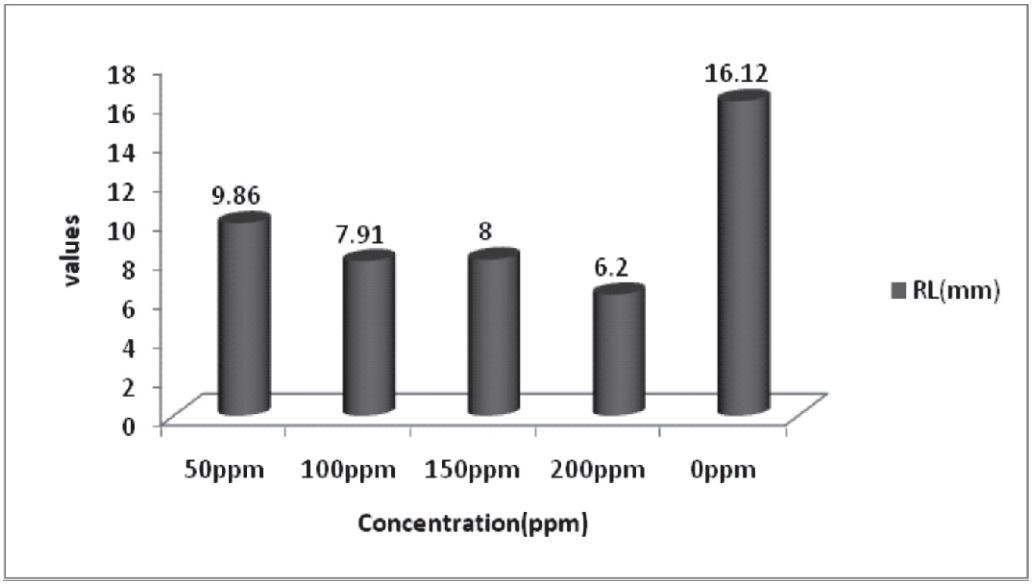

Fig. 4.2: Radicle Length of Phaseolus vulgaris
LR = Radicle Length.

TABLE 4.3: Germination Parameters of *Vigna unguiculata* seeds soaked in Different Concentration of IAA

Parameters	50ppm	100ppm	150ppm	200ppm	0ppm
MGT (day)	1.55	2.00	1.56	2.00	2.00
GRT(%/day)	26.67	21.67	21.66	14.17	12.50
FDG (day)	1	2	1	1	1
LDG (day)	2	2	2	4	4
TSG (day)	1	0	1	3	3
FGP (%)	36.67	43.33	30	23.33	20

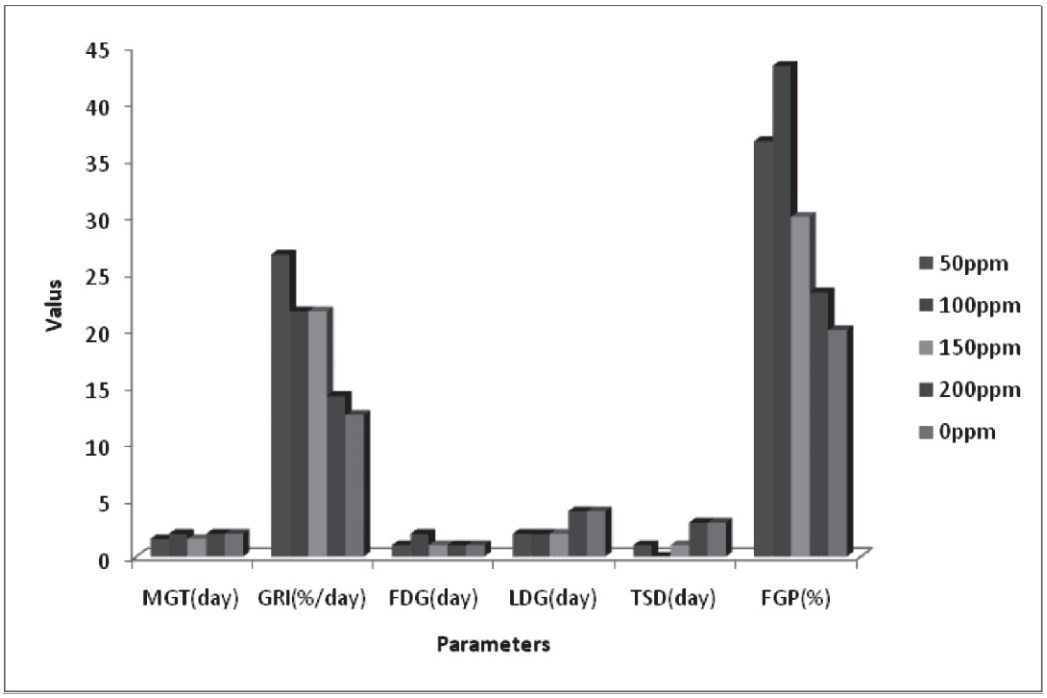

Fig. 4.3: Germination parameters of *Vigna unguiculata* seeds

MGT= Mean germination time, GRT = Germination rate time, FDG = Final day of germination, LDG = Last day of germination, TSG = Time spread of germination, FGP = Final germination percentage

TABLE 4.4: Radicle Length of *Vigna unguiculata*

Parameters	50ppm	100ppm	150ppm	200ppm	0ppm
Radicle length(mm)	6.67	7.33	5.89	6.11	7.00

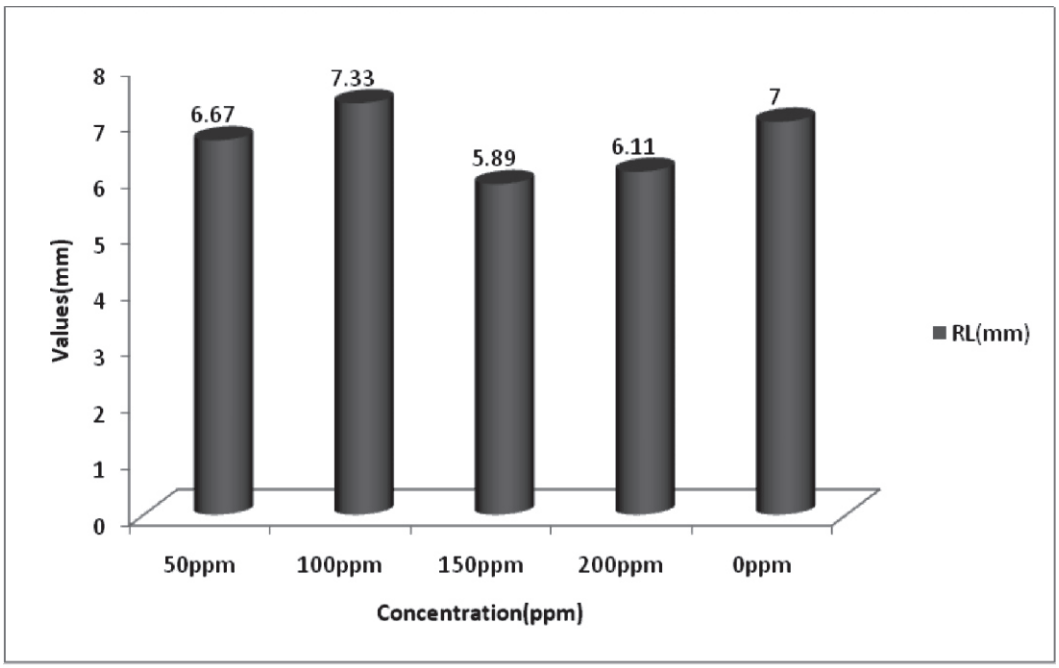

Fig. 4.4: Radicle Length of *Vigna unguiculata*
LR = Radicle Length.

DISCUSSION

Priming of seeds has proven to be efficient in increasing rate of seed germination and seedling growth (Basra *et al*. 2003). In this study, two varieties of cowpea *Vigna unguiculata* and *Phaseolus vulgaris* were primed to determine the effect of IAA on their germination. The Mean Germination Time was least in 0ppm for *Phaseolus vulgaris* and 50ppm for *Vigna unguiculata.* This indicates that more seeds germinated faster in 0ppm of *Phaseolus vulgaris* and *Vigna unguiculata* than the other treatments. The two concentrations were able to enhance germination by affecting their metabolic activities. According to Ashrafi and Razmjoo (2010) and Basra *et al*. (2006), seed priming is pre-sowing strategy to affect the pre-germination metabolic activities to enhance the germination. Similarly, the value of Germination Index was indicated that the highest value was in 0ppm for *P. vulgaris* and 50ppm for *V. unguiculata* respectively. This showed that more germination occurred per day in both concentrations for the cowpea. On the contrary, least germination per day was recorded in 200ppm for both Varieties of cowpea. This indicates that high concentration of IAA have the ability to inhibit germination of seeds.

Furthermore, the highest Germination Percentage was recorded in 0ppm (80%) in *P. vulgaris* and 100ppm (43.33) in *V. unguiculata.* In *V. unguiculata* none of the treatments recorded up to 50% germination. This could be a physiological or time factors associated with the *V. unguiculata* as IAA was shown in breaking dormancy of the seeds. But in *P. vulgaris*, except in

150ppm (40%) and 200ppm (13.33%), other IAA concentrations had more than 50% germination. Apart from 200ppm in *P.vulgaris* and 100ppm in *V. unguiculata* where first germination occurred 2 days after planting, other concentrations record first germination 1 day after germination. Here, the period for first germination to occur in the two cowpea varieties is independent of the concentration or the growth hormone used. The minimum Last day of Germination was 2 days in both varieties while the maximum was 4 days. As in First Day of Germination, Last Day of Germination seems not to be affected by concentration gradient. The germination of these Varieties of cowpea indicates that germination per meters measure was not influenced by differences in IAA concentration but Variety of species. This is in line with the findings of Guan (2009) in different varieties of maize studied.

The radicle length was recorded to be highest in 0ppm and 100ppm among the *P. vulgaris* and *V. unguiculata* of cowpea studied.

CONCLUSION

The results of this work revealed that IAA generally has little effect on *P. vulgaris* and little or no effect on *V. unguiculata* on germination. Therefore could not be a good priming material for them.

RECOMMENDATION

Cowpea been an important crop to both human and livestocks, need an improvement in its production. I, therefore suggest more work on other growth hormones to determine which is better for its priming and production.

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