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Short Communication

Effect of fungicide on the vegetative parameters of green gram (*Vigna radiata L Wilczek*)

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Abstract

This experiment was conducted in laboratory scale to study on the effect of fungicide (sulphur) on the vegetative parameters of green gram. For that Green gram seeds are subjected at different concentration of elemental sulphur powder which is used as a fungicide to study the germinations and vegetative parameters like root length, shoot length, root and shoot fresh weight and dry weight and chlorophyll content. It is observed that rate of germination 27.9% of seeds were found to be germinated after 24hour in the controlled condition. However 3.7% of green ground seed germination were found in 5.0g/lt concentration of sulphur under control condition root length of phaseolus aureus var PDM_{54} seedlings are 5.4±0.04cm where as treatment of 5.0g/lt concentration of sulphur is 1.6±0.01 root lengths were observed. Under control condition shoot length 14.2±0.10 of phaseolus aureus var PDM_{54} where as seedlings are 6.3±0.04cm treatments of 5.0gm/lt concentration of sulphur, 1.6±0.01 root lengths were observed.

Keywords: Fungicide, green gram, sulphur, chlorophyll, germination

Introduction

Green gram (*Vigna radiata L. wilczek*) is a principal important short duration and drought tolerant pulse crop in India. It is a leguminous species grown principally for its protein-rich edible seeds and also has a potential as a green manure as a forage crop. The productivity of *Vigna radiata (L.) Wilczek* in summer as influenced by different showing dates and varieties[1]. Pulses are the major food for the human welfare for maintaining proteinous nutrient. In India area occupied by green gram is about 23.63 million hector. With total production of 14.56 million tones but average productivity is 625kg/ha., which is quite low[2]. However the pulse is destructed due to attack of different types of fungi, so that a number of synthetic pesticides are used by the farmers, but that causes to the pollutions in the environment. Biological pesticides causes eco friendly to the environment. Biocides are causes good vegetative parameters yields, macro molecules contents like chlorophyll, carbohydrate, protein, amino acids and nucleic acids contents also increases in lower concentration but decreases in higher concentration of pesticides. The decapitation on yield of green gram is given by Seran et. al. shows that at the 3rd week after planting would be more effective to obtain high yield[3]. Influence of single spray of 0.5% $FeSO_4$ at 25 DAS recorded grain yield 716.76 kg/ha and in 45DAS it is 570kg/ha shows that the treatment of $FeSO_4$ increases the yield[4]. Santoshkumar et. al. reported that chlorpyrifos is reduces the green gram seed germination percentage decrease significantly when increasing of concentration of insecticide[5].

Green gram contains around 21-23% of protein[6]. Application of 100% NPKS along with bio-fertilizer significantly increase the growth of plant and yield[7]. Sulphur is a yellowish-grey wettable powder widely used as a fungicide to control powdery mildews on chilli, mango, pea, ber etc. and also as an acaricide to control mites on tea, cotton, mango, jowar, roses, cucurbits etc., and also as an acaridcide to control mites on tea, cotton, mango, jowar, roses, cucurbits etc. and powder mildewsapes, groundnut, beans, bhendi, bottle guards; mode of application-wet method. Although irritation to eyes and skin, sulphur has low toxicity to mammals. Sulphur consisted of four levels through four sources i.e. elemental sulphur, mahadhan bensulf, pyrite and gypsum. The nutritive value of pulses is determined by the proportion of sulphur containing amino acids which not only improve the quality of the produce but also increase the yield[8,9]. The decrease in yield arises due to sulphur deficiency caused by intensive agriculture, use of high yield rice verities, use of sulphur free fertilizers and decreased use of organic wastes, Sulphur deficiency is more common under low land ecosystem for rice. Sulphur deficiency decreases the synthesis of proteins, vitamins and sulphur containing amino acids and is associated with N-metabolism. Sulphur dioxide caused air pollution and adversely affects plants in various forms, exposure of crop plant to SO_2 at detrimental levels cause's leaf chlorosis and necrosis, retarded photosynthesis, altered metabolic parameters including change in some enzyme activities. The morpho-physico injuries in crop plant industrially polluted areas caused by pollutants relate their concentration and duration of exposure.

Sulphur has unique property of acting as an oxidizing and reducing agent and SO_2 is a competitive inhibitor in carbon assimilation and interferes in mitochondrial ATP production[10]. The effect of phospho-gypsum is given by Banik et. al and the result reveals that application of phospho-gypsum at 30kg S/ha with the recommended dose of N-P-K gives highest yield[11]. The effect of phosphorus and sulphur on growth, yield and economics of green gram is given by Punse et. al[12] and Patil et. al[13]. The effect of phosphorus and sulphur on growth and yield during Kharif season of 2016 is given by Serawat et.al and reported that application of 30kg $P_2O_5ha^{-1}$ and 20kg S ha^{-1} significantly increased the total leaf chlorophyll content[14]. From the previous study it is clear that application of sulphur and phosphorus compounds in soil gives good result[15-18], however application of elemental sulphur as a fungicide has some limitations. Phyto injuries in plant species are too complicated to assess as they are totally affected by the ecosystem and the consideration of influences of air pollutants.

Materials and methods

The present investigation was studied the "Effect of fungicide on the vegetative parameters of green gram was conducted in the research laboratory. Good healthy variety of Phaseolus aureus var. PDM_{54} was collected and sulphur powder is used as fungicide while seed germination and seedling growth experiments were conducted in the laboratory. The experiments for study vegetative growth and yield parameters were carried out in the departmental garden by pot culture method. 1000 numbers of green grams are taken for germination study. All the glass wares including seeds have been properly sterilized. Prior to germination, all the seeds were surface sterilized by treating with 5% concentration of sodium hypo chloride solution for 12 minutes and then thoroughly washing it with distilled water for 3 to 4 times to remove completely the sodium hypo chloride solution. Glass materials, absorbent cotton, filter papers and vermiculites were properly sterilized and used for germination of green gram. Glass wares that used in this experimental study were made up of corning/borosil glass and after a proper cleaning; they were placed in an incubator maintaining 90^0C to avoid fungal infection.

Cultivation of System: Germination bed: Different concentration of Sulphur (0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0,4.5,5.0 gm/liter) visually selected and surface-sterilized seeds of all the 3 test crops were allowed to germinate in petri dish having each 10cm in size, 50 seeds Petri dish lined with blotting paper. A set of 20 Petri dishes containing total number of 1000 seeds was prepared for each concentration of the test chemical. Control set of Petri dish were prepared by taking similar amount. Watering was allowed in all the cases at an interval of 12 hrs, amounting 7CC. in each Petri dish.

Germination Chamber: Specially designed germination chamber was used for germination of test seeds under different concentration of test chemicals.

Light is an inhibitory factor for the process of the germination, hence precaution were taken to make the germination chamber light proof throughout the course of investigation. Thermo stability maintenance during germination was made keeping the mercury label at 25^0C. The duration of germination experiment limited to 72 hrs. First observation of germination was recorded after 12 hrs. Treatment and subsequently at an interval 12 hrs. duration the germination strategy of scheduled i.e. 72 hrs. The course of germination experiments adequate ventilation was allowed under the laboratory conditions and time to time administration of water with test chemicals was carried on to facilitate the germination parameters.

Seedling chamber: A special type of chamber has been designed for the growth of germination seed designated as seedling chamber. The chamber is partitioned by wooden plates to keep the Petri dish besides maintaining of thermo-stability, photo-stability has been allowed with 2000±200 lux of fluorescent light throughout the course of seedling growth. Temperature constancy maintained at 25^0C during the course of seedling growth investigations. Petri dishes with germination seeds were transferred to the seedling chamber. Growth of radical and plumule of the germinating seeds were allowed for seven days. Throughout the period of seedling growth aeration under the laboratory conditions was kept intact. Random collection of seven days old seedlings was made from the seedling chamber for the different physiological and biochemical experimental investigations.

Physiological Methodology: Root Length: The primary roots resulted from growth of the radical of seven day old seedlings were taken for physiological analysis related to the controlled and experimental conditions, Random selection of 100 samples were made for finding the longitudinal growth of the tap root. Mean length of the roots were calculated and statistical analysis was made.

Shoot length: Elongation of plumule through the micropyle results the shoot systems. Longitudinal growth of the shoot of seven day old seedlings has been measured. The random selection of 100 samples was made for the purpose. Statistical analysis was then carried on to test the validity of experiment.

Extraction and estimation of chlorophyll: In order to measure the chlorophyll pigments (chl.-a, chl.-b and total chlorophyll) in leaves, ten seedlings were taken of randomly from each Petri dish of both control and treated assets at an interval of seven days from 7 to 35 days; leaves were separated from seedlings, washed thoroughly, dried the surface water by soaking on a blotting paper, cut in to small pieces and weighted for 50mg. then this weighted samples were homogenized with 1-2ml. of 80% ethanol (v/v) with mortar and pestle, the homogenate were centrifuged at 3000 xg for 20 minutes at 28±10c. The pellets were again homogenized with 80% ethanol and centrifugation was repeated till colorless pellets obtained which were further used for other chemical analysis.

All the supernatants were pooled together and a total volume of 10ml was prepared with 80% ethanol. The optical density (O.D.) of the extract was measured with help of Elico digital spectrophotometer (model CL-27) at 663, 645nm taking 80% ethanol as blank pigment estimations were calculated following the formulae suggested by Arnon (1949).

$$\text{Chlorophyll-(a)}= \frac{12.7\times(\text{O.D.at663nm})-2.69\ \text{X}\ (\text{O.D.at645nm})}{1000\times W} \times \text{vmg/g (fresh weight)}$$

$$\text{Chlorophyll-(b)}= \frac{22.9\times(O.D.at645nm)-4.68\times(O.D.at663nm)}{1000\times W} \times \text{vmg/g (fresh weight)}$$

$$\text{Total Chlorophyll}= \frac{20.2\times(O.D.at645nm)+8.02\times(O.D.at663nm)}{1000\times W} \times \text{vmg/g (fresh weight)}$$

Where, V= Total volume of extract in C.C, W= Weight of samples in gram, O.D. = Optical density of the extract.

Results and discussion

In the controlled and test chemical treatment condition shows variability in the rate of germination 27.9% of seeds were found to be germinated after 24hours. However 3.7% of green ground seed germination were found by using 5.0g/lt., concentration of sulphur. Under control condition root length of Phaseolus aureus var PDM_{54} seedlings are 5.4±0.04cm where as treatment of 5.0g/lt., concentration of sulphur , 1.6±0.01 root length were observed. Under control condition shoot length 14.2±0.10 of phaseolus aureus var PDM_{54} where as seedlings are 6.3±0.04cm while treatment of 5.0g/lt., concentration of sulphur 1.6±0.01 root length were observed. Under control condition chlorophyll content 4.39±0.08 of phaseolus aureus var PDM_{54} where as seedlings are 1.93±0.03cm treatments of 5.0mg/lt. concentration of sulphur, 1.6±0.01 root length was observed.

Generally height of the plant increases due to inter nodal elongation regulated by plant growth substances such as gibberellins and auxins In pulses, generally the component of yield comprises, the production of number of pods per plant, weight of 1000 seeds etc.

The productions of number of pods are resulted by development of flowers per inflorescences, proper micro and micro and mega spore genesis fertilization. All these process are generally controlled by both external environmental factor and internal genetic characters. The synthesis of endogenous growth regulators responsible for flower formation is controlled by environmental factor such as solar radiation, nitrogen level, water supply. The germination potency of phaseolus auries var. PDM_{54} at different concentration of sulphur is given in Figure-1 and effect of sulphur on vegetative parameters and chlorophyll content of phaseolus auries var PDM_{54} are given in Table-1.

Seedling establishment is considered as one of the crucial stage. After germination, where various morphogenesis activities are established along with changes in physiological and biological processes. During this period the seedlings try to become self established by the development of root and shoot systems. The growth and development of shoot and root are controlled by various metabolic activities carried out in leaves; hence changes in shoot and root length of seedlings and their fresh and dry weights, changes in macromolecular contents such as chlorophylls, carbohydrates, proteins and nucleic acid are considered important parameters of seedling establishment. All the concentration of considerably reduced the seedling growth due to interaction of phytotoxins with photo phosphorylation pathway and inhibition of light activated for phase activities. Though the carbohydrate synthesis mainly depends on the efficacy of photosynthetic processes, the accumulation of carbohydrate in plants is directly proportional to the amount of chlorophyll present in the leaves. All concentration of above test chemicals gradually exhibited a steady state of declination in protein content in all the test crops might be due to the cultivation of synthesized proteins in the formation of same hydrolytic enzymes. The synthesis of nucleic acids (DNA and RNA) is also direct related to protein, carbohydrate and other metabolic contents in seedlings might be due to lower range of other metabolites.

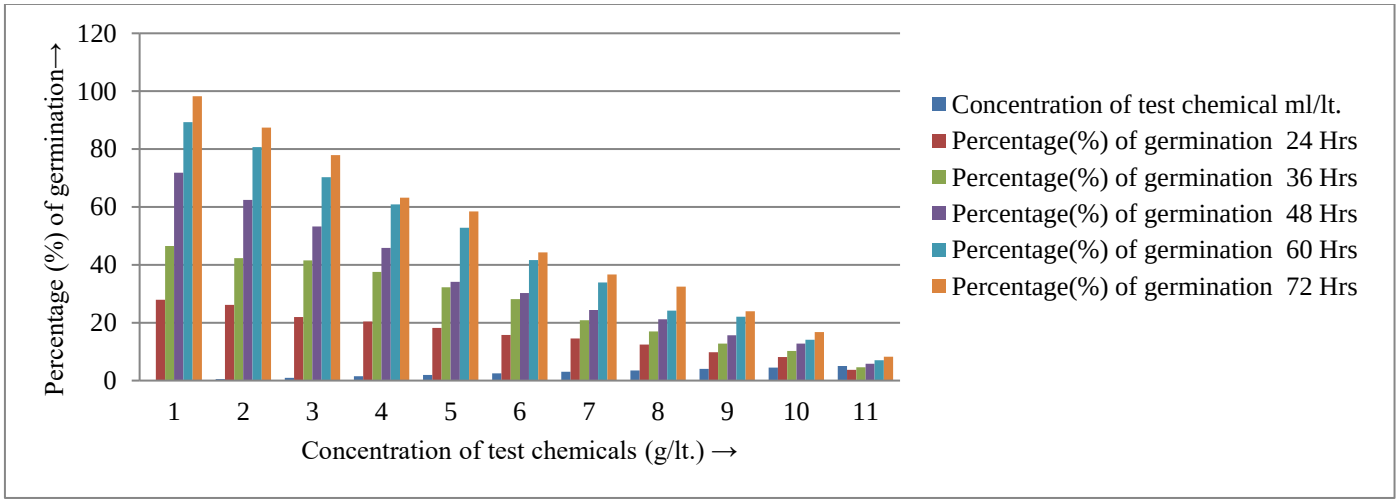

Figure-1: Germination potency of Phaseolus aureus var. PDM_{54} at different concentration of Sulphur.

Table-1: Effect of sulphur on vegitative parameters and chlorophyll content of Phaseolus auries var PDM_{54}.

Treatment (ml/lt.) Parameter`s		Control	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Shoot length(cm)		14.2± 0.10	13.4± 0.009	12.8± 0.09	12.1± 0.09	10.2± 0.08	9.6± 0.07	8.7± 0.06	8.5± 0.05	7.6± 0.05	6.8± 0.04	6.3± 0.04
Shoot fresh weight (mg)		185.4± 0.23	169± 0.21	158.2± 0.20	145.3± 0.19	129.3± 0.17	114.7± 0.16	102.8± 0.15	95.8± 0.14	81.7± 0.13	58.4± 0.01	46.7± 0.09
Shoot dry weight(mg)		36.3± 0.11	32.6± 0.11	30.5± 0.10	26.4± 0.10	23.8± 0.09	18.7± 0.08	16.4± 0.08	13.7± 0.08	11.8± 0.07	9.6± 0.06	6.2± 0.05
Root length (cm)		5.4± 0.04	4.9± 0.03	4.6± 0.03	4.4± 0.03	3.6± 0.02	3.1± 0.02	2.8± 0.02	2.6± 0.01	2.3± 0.01	2.1± 0.01	1.6± 0.01
Root fresh weight(mg)		49.1± 0.09	46.8± 0.09	43.1± 0.09	36.7± 0.08	33.8± 0.08	28.0± 0.07	23.0± 0.07	21.3± 0.07	18.7± 0.13	15.8± 0.06	14.5± 0.06
Root dry weight(mg)		8.6± 0.04	7.3± 0.04	7.2± 0.04	6.7± 0.03	6.2± 0.03	5.6± 0.03	5.2± 0.03	4.8± 0.02	4.3± 0.02	3.5± 0.02	3.1± 0.02
Chlorophyll content mg/g	Chl.-a	3.45± 0.08	3.12± 0.07	3.06± 0.07	2.84± 0.07	2.62± 0.06	2.37± 0.05	2.24± 0.05	1.93± 0.04	1.82± 0.04	1.62± 0.04	1.51± 0.03
	Chl.-b	0.94± 0.04	0.87± 0.04	0.82± 0.04	0.76± 0.03	0.68± 0.03	0.62± 0.03	0.58± 0.02	0.54± 0.02	0.49± 0.02	0.46± 0.02	0.42± 0.02
	Tot.chl.	4.39± 0.08	3.99± 0.07	3.88± 0.07	3.6± 0.07	3.30± 0.06	2.99± 0.05	2.82± 0.05	2.47± 0.04	2.31± 0.04	2.08± 0.04	1.93± 0.03

Conclusion

The different concentrations of the test chemicals (0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0,4.5,5.0g/lt) inhibited the seed germination process at 5.0 g/lt. concentration. The lower concentration of biocides caused higher rate of seed germination, but higher concentration exhibited negative correlation with germination process. The parameters of seedling growth, fresh and dry weight of both shoot and root and contents of macro molecules like chlorophyll contents were significantly inhibited.

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