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Impact of Salicylic and Gibberellic Acid Seed Invigoration on Seed Health Status under (Artificial Ageing) Accelerated Ageing Test in Lentil (*Lens culinaris* Medik.)

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ABSTRACT

Background: Research examination led during the *Rabi* season of 2018-19 and 2019-20 to discover the Effect of hormonal seed strengthening on seed health status under accelerated ageing test in lentil (*Lens culinaris* Medik.) at state seed testing laboratory. The Blotter test was conducted in CRD plan with four replications. From the examination it was demonstrated that the hormonal treatment with salicylic acid at RH (90%) and temperature (35°C) for 2 days ageing showed least infection, while contagious contamination higher with the increment in relative humidity, temperature and duration of artificial ageing.

Methods: During the years 2018 to 2020, the current experiment was carried out at the state seed testing laboratory, Department of Genetics and Plant Breeding, SHUATS, Prayagraj, Uttar Pradesh. In the experiment, 37 different treatment permutations were used. To accelerate the ageing process, the seeds were placed in an ageing chamber with high temperatures and relative humidity. The aged seeds was then revitalized with hormones, a process known as hormonal seed fortification.

Result: Our finding revealed that the aged seeds treated with gibberellic acid and salicylic acid (Growth Hormone) enhanced the quality of seed health status in Lentil.

Key words: Accelerated ageing, Blotter test, Hormonal seed invigoration, ISTA techniques, Seed health.

INTRODUCTION

Pulses are a significant commodity crop group that provides high-quality protein to supplement cereal protein for the country's predominantly vegetarian population. Despite being the world's largest pulse crop cultivating country, India's pulse production pales in comparison to total cereal crop production. Pulses cultivation develops a mechanism to fix atmospheric nitrogen in their root nodules, allowing them to fulfill their nitrogen needs to a large extent.

Lentils is a diploid species (2n = 14) (Muehlbauer, 1991). It is self-pollinating annual species. It is the oldest cultivated legume plant (Bahl *et al*., 1993; Rehman *et al.,* 1994). Lentil (*Lens culinaris* Medik.) is one of the world's oldest grown and consumed annual grain legumes. As early as 6000 B.C., they came from South Western Asia. Its origin is the near East and Egypt at the Central and Southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India and Pakistan, China and later spread to Latin America (Cubero 1981, Duke 1981). Lentil in taxonomy is as follows: Kingdom Plantae-Plants, Subkingdom Tracheobionta-Vascular plants, Superdivision Spermatophyta-Seed plants, Division Magnoliophyta-Flowering plants, Class Magnoliopsida-Dicotyledons, Subclass Rosidae, Order Fabales, Family Fabaceae-Pea family, Genus Lens Mill-lentil, Species *Lens culinaris* Medik-lentil (Anonymous, 2012).

Nutritional value of lentil per 100 g dry weight are energy 1,477 kJ (353 kcal), carbohydrates 60 g, sugars 2 g, dietary fibre 31 g, fat 1 g, protein 26 g, vitamins thiamine (B1) (76%) 0.87 mg, riboflavin (B2) (18%) 0.211 mg, niacin (B3) (17%) 2.605 mg, pantothenic acid (B5) (42%) 2.120 mg, Vitamin B6 (42%) 0.54 mg, Folate (B9) (120%) 479 µg, Vitamin C (5%) 4.4 mg, Trace metals *i.e*., Calcium (6%) 56 mg, Iron (58%) 7.54 mg, Magnesium (34%) 122 mg, Phosphorus (64%) 451 mg, Potassium (20%) 955 mg, Sodium (0%) 6 mg, Zinc (50%) 4.78 mg, Other constituents Water 10.4 g. (Source: USDA Nutrient Database). Delouche (1965) first presented accelerated ageing as a test for seed quality at the seed innovation research facility, Mississippi State University, USA. Initially, it was created as a test to assess the strength of seeds in warehouse storage. Ensuing examinations have affirmed the precision of this test in anticipating the lifetime of a wide range of types of seeds inside the storage range (Delouche and Baskin, 1973).

Seed invigoration alludes to any post collect treatment that further develops seed force, bringing about further

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developed germinability, storability and field execution over the coordinating untreated (control) seed. Further developing seed quality is a methodology that is probably going to yield huge advantages in practically all conditions while having no huge danger. Seed enhancement procedures are not new to horticulture and past approaches for comparable medicines have been accounted (Kalyani *et al.,* 2009).

Hormonal seed invigoration assumes a significant part in seed metabolism (Rhaman *et al*., 2020). As of now, hormonal seed treatment is a generally utilized strategy to further develop seed germination, seedling development and harvest yield in unfavorable conditions. Guaranteeing better germination and seedling life by seed preparing would bring about healthy and useful plants under antagonistic conditions (Hasanuzzaman and Fotopoulos 2019; Hu *et al*., 2013).

Seed health testing to identify seed-borne microorganisms is a significant stage in the administration of harvest infections. Seed health is a proportion of opportunity of seeds from microbes. Particularity, affectability, speed, effortlessness, cost adequacy and unwavering quality are principle prerequisites for determination of seed wellbeing tests strategies. Seed wellbeing testing strategies can be applied to comprehend the wellsprings of seed borne contaminations, area of microorganisms inside seed tissues, to affirm the event of seed transmission and its components and to comprehend the impact of outer biotic and abiotic factors on seed transmission or different periods of the disease cycle (Tsedaley, 2015). Seed health is a superior indistinguishable component in the agricultural science for desired plant population and great harvest (Rahman *et al*., 2008). Seed borne microbes are a tremendous issue and may even be liable for the re-bulge of sicknesses of the past just as the execution of illnesses into new areas (Gitaitis and Walcott, 2007). Seed borne microbes present an extreme danger to seedling creation (Walcott, 2003). In the present period, seed accounts like never before for the development of plant microbes across huge distances, normal boundaries and political lines (Gitaitis and Walcott, 2007). Parasites outnumber any remaining kinds of microorganisms that assault plants and cause an intense financial effect on agrarian creation because of their capacity to carry out sicknesses of developed harvests that outcome in yield misfortunes (Paplomatas, 2006).

Seed serve as method for dispersal and endurance of plant micro-organisms. It is basic to test its wellbeing prior to utilizing it as establishing material. Seed wellbeing testing and identification is a first line approach in overseeing seed borne infections of plants. The investigation of seed-borne microorganisms is important to decide seed wellbeing and to further develop germination capability of seed which at last prompts increment of the yield creation. In connection with those significance of seed wellbeing tests and discovery of seed borne microbes; the present investigation research experiment has been planned with the objective to know the impact of hormonal seed invigoration on the seed health status under accelerated ageing in lentil seeds.

MATERIALS AND METHODS

The experiment was conducted at state seed testing Laboratory of the Department of Genetics and Plant Breeding, SHUATS, Prayagraj during 2018-2020. Lentil seeds (*var*. K-75) were obtained from Directorate of seed and farm NAI, SHUATS, Prayagraj, Uttar Pradesh. For conducting accelerated ageing (artificial ageing) a delicate muslin cloth was knotted around 100 seeds in four replications. On a wire mesh, the tied seeds were placed in a jar. The jar's lower half was filled with water. Water should not come into direct contact with the seed. To make it airtight, the jar was covered with the lid and sealed with parafin wax. After that, the jar was placed in an accelerated ageing chamber that was kept at 35-40$^{238}_{92}$C temperature and relative humidity for 0, 2,4 and 8 days. After this time, the container was removed and the seeds were chilled in a desiccator. (Delouche and Baskin 1973).

For the preparation of solution one mg of each chemical was taken in a beaker. These chemicals were added separately in 1000 ml. of distilled water with constant stirring. The volume of solution was finally constituted to one litter and then it became 1000 ppm stock solution of each chemical. The flasks containing chemicals was covered with muslin cloth to avoid any contamination. For the preparation of GA_3 (100 ppm) and Salicylic acid (100 ppm), 100 mg of GA_3 and Salicylic acid was taken in a measuring flask and made up to 1000 ml. distilled water. After preparation of solution of GA_3 and Salicylic acid, lentil seeds (aged seed) were soaked in required solution for 12 hours at 25$^{238}_{92}$C temperature. After 12 hrs of soaking the solution was drained out from the beaker and pre-soaked air dried to original weight and then placed for blotter test in laboratory under controlled condition.

Blotter test was done according to (ISTA, 2010). Seed health testing for fungal infection was carried out using blotter technique for each sample. Ten seeds in four replicates were placed equidistantly on three layered sterile blotter paper moistened with distilled water in sterile Petri plates under aseptic condition and incubated at 20±2$^{238}_{92}$C for 7 days with alternate cycles of 12 h in near ultraviolet light (NUV) range and for the remaining 12 h in dark. On the eighth day, the seeds were examined for the presence of fungal infection. The numbers of infected seeds were counted and the mean value was expressed in percentage. Blotter tests are similar to germination tests in that seeds are placed on moistened layers of blotter paper and incubated under conditions that promote fungal growth.

Statistical analysis

Experiments were replicated four times and results on the percentage fungal incidence and percentage germination of seeds on the treatment were analyzed using CRD. The mean sum of squares due to treatments showed significant difference for all combination of treatment under study at 5% level of significance.

The analysis of variance was worked out to test the significant differences among the treatments by F- test. It was carried out according to the procedure of complete block design for each character as per methodology suggested by Fisher (1936).

RESULTS AND DISCUSSION

The hormonal treatment with salicylic acid at RH (90%) and temperature (35°C) for 2 days ageing showed minimum fungal infection, whereas fungal infection higher gradually with the increase in relative humidity, temperature and

Table 1: Mean performance for seed health status due to the effect of seed invigoration under accelerated ageing in lentil (*Lens culinaris* Medik.).

S. no.	Treatments notation	1st Year (2018-19)	2nd Year (2019-20)	Pooled data
T_0	Control	1.25	1.37	1.31
T_1	$H_1T_1D_1$	1.40	1.87	1.64
T_2	$H_1T_1D_2$	1.48	2.13	1.81
T_3	$H_1T_1D_3$	1.60	2.46	2.03
T_4	$H_1T_2D_1$	1.66	2.63	2.14
T_5	$H_1T_2D_2$	1.71	2.64	2.17
T_6	$H_1T_2D_3$	1.69	2.67	2.18
T_7	$H_2T_1D_1$	1.78	2.75	2.26
T_8	$H_2T_1D_2$	1.58	2.79	2.18
T_9	$H_2T_1D_3$	1.87	2.83	2.35
T_{10}	$H_2T_2D_1$	1.87	2.83	2.35
T_{11}	$H_2T_2D_2$	1.93	2.86	2.39
T_{12}	$H_2T_2D_3$	2.43	2.88	2.65
T_{13}	$P_1H_1T_1D_1$	0.58	0.54	0.56
T_{14}	$P_1H_1T_1D_2$	0.43	0.65	0.54
T_{15}	$P_1H_1T_1D_3$	0.90	0.60	0.75
T_{16}	$P_1H_1T_2D_1$	0.98	0.86	0.92
T_{17}	$P_1H_1T_2D_2$	0.93	0.87	0.90
T_{18}	$P_1H_1T_2D_3$	1.14	0.90	1.02
T_{19}	$P_1H_2T_1D_1$	0.69	0.95	0.82
T_{20}	$P_1H_2T_1D_2$	0.64	1.06	0.85
T_{21}	$P_1H_2T_1D_3$	1.41	1.11	1.26
T_{22}	$P_1H_2T_2D_1$	1.09	1.39	1.24
T_{23}	$P_1H_2T_2D_2$	1.64	1.62	1.63
T_{24}	$P_1H_2T_2D_3$	2.01	2.00	2.00
T_{25}	$P_2H_1T_1D_1$	0.39	0.35	0.37
T_{26}	$P_2H_1T_1D_2$	0.88	0.39	0.64
T_{27}	$P_2H_1T_1D_3$	0.53	0.49	0.51
T_{28}	$P_2H_1T_2D_1$	0.86	0.83	0.84
T_{29}	$P_2H_1T_2D_2$	1.21	0.97	1.09
T_{30}	$P_2H_1T_2D_3$	1.17	1.15	1.16
T_{31}	$P_2H_2T_1D_1$	1.54	1.19	1.36
T_{32}	$P_2H_2T_1D_2$	1.69	1.34	1.51
T_{33}	$P_2H_2T_1D_3$	1.00	1.52	1.26
T_{34}	$P_2H_2T_2D_1$	1.63	1.61	1.62
T_{35}	$P_2H_2T_2D_2$	1.36	1.67	1.52
T_{36}	$P_2H_2T_2D_3$	1.73	1.72	1.72
Grand mean		1.32	1.58	1.45
S. Ed. (±)		0.18	0.01	0.09
SE(m)		0.15	0.01	0.07
C. D. (P = 0.05)		0.46	0.04	0.22

Where,

H1= 90% Relative humidity, H2= 100% Relative humidity.

D1= 2 days, D2= 4 days, D3= 8 days.

T1= 35°C temperature T2= 40°C temperature.

P1= Gibberellic acid (100 ppm), P2= Salicylic acid (100 ppm).

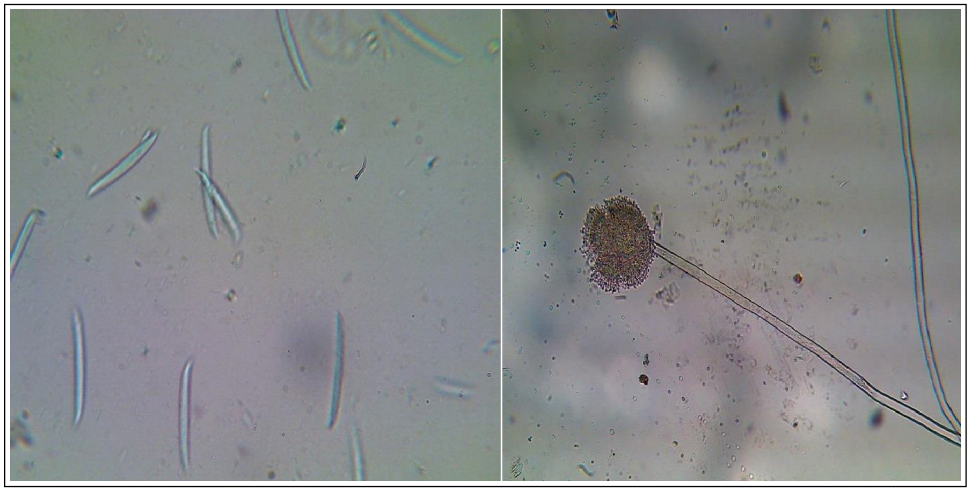

Plate 1: Appearance of fungal infection *Fusarium* and *Aspergillus* species during blotter test in artificial aged seed.

duration of accelerated ageing in both years. The mean value for seed health recorded for all treatments were (1.32), (1.58) and (1.45) respectively.

All through the experiment, the fungal infection varied significantly among the treatments. The lowest fungal infection was recorded in seeds treated with T_{25} notation $P_2H_1T_1D_1$ [Salicylic acid +RH (90%)+Temp. (35°C)+Days (2)] (0.37%) at par with T_{27} notation $P_2H_1T_1D_3$ [Salicylic acid+RH (90%)+Temp. (35°C)+Days (8)] (0.51%), T_{14} notation $P_1H_1T_1D_2$ [Gibberellic acid+RH (90%)+Temp. (35°C)+Days (4)] (0.54%) and T_{13} notation $P_1H_1T_1D_1$ [Gibberellic acid +RH (90%)+Temp. (35°C)+Days (2)] (0.56%) followed by T_{26} notation $P_2H_1T_1D_2$ [Salicylic acid+RH (90%)+Temp. (35°C)+Days (4)] (0.64%) and T_{15} notation $P_1H_1T_1D_3$ [Gibberellic acid+RH (90%)+Temp. (35°C)+Days (8)] (0.75%) and while significantly highest infection was recorded in T_{12} notation $H_2T_2D_3$ [RH (100%)+Temp. (40°C) + Days (8)] (2.65%) under accelerated ageing conditions.

Our results showed that the application of salicylic acid and gibberellic acid had a signal suppressive influence on the spore germination of pathogens and mild suppressive effect on spore, salicylic acid germination and growth rate of colony. Our result parallel to those of the studies (Hayat *et al*., 2012), (Wasternack *et al*., 2017); (Degani *et al*., 2015); (Klessig *et al.,* 2018) (Ferrari *et al*., 2003; Li and Zou, 2017). (Mishra *at al.,* 2019) suggested that the Gibberellic acid and salicylic acid has many impacts, for example, animating the plant to frame protein-related with pathogenesis and expanding the blossoming time frame, represses the arrangement of ethylene, developing the seeds and containing wounds. SA had the option to essentially lessen *F. oxysporum* development, since SA is a characteristic phenolic compound that have inhibitory impact on microbial and that the motivation to poisonous impact on parasite**.** SA is a guard related plant chemical that assumes a vital part in protection from various microbial microorganisms, like infection, microscopic organisms, parasites and oomycetes (Kunkel and Brooks, 2002; Vlot *et al*., 2009). In plants, the positive connection between endogenous degrees of SA and opposition reactions against biotrophic and hemi biotrophic microbes are grounded (Glazebrook, 2005). In addition, the exogenous SA application initiates nearby and foundational gained opposition in various plant species against different kinds of microorganisms, including *Fusarium oxysporum*, *Alternaria alternata*, *Magnaporthe grisea*, *Colletotrichum gloeosporides*, *Xanthomonas* spp., different kinds of viruses. Arising proof showed that parts of GA flagging assuming a huge part in the resilience and weakness of plant sicknesses (Robert-Seilaniantz *et al*., 2011). SA isn't just controlling the protection from biotic burdens, yet additionally the resilience to different abiotic stresses (Horváth *et al*., 2015; Khan *et al*., 2015). The physiological and biochemical quality characteristics of seeds were significantly impacted negatively by ageing (Patil *et al*., 2021).

It was seen that there is significant variation among the treatments. The anova table showed that the calculated values of F due to the effects of invigoration under accelerated ageing were higher than the respective table values at 5 %, so null hypothesis will be rejected. As shown in Table 1 the average performance for seed health status due to the impact of seed invigoration under accelerated ageing in Lentil (*Lens culinaris* Medik.). The hormonal seed fortification with plant growth regulators (Salicylic acid) for 2 days of accelerated ageing at 90% relative humidity and 35°C temperature demonstrated minimal fungal infection, but fungal infection increased steadily with increasing relative humidity, temperature and length of accelerated ageing.

Plate 1 detailed about detection of fungal infection *i.e*., *Fusarium* and *Aspergillus* species during blotter test in artificial aged seed.

CONCLUSION

From the present investigation it is concluded that the salicylic acid was more effective to stop fungal contamination in aged seed in comparison to Gibberellic acid. Salicylic

acid is a phenolic compound that have inhibitory impact on microbial and that the motivation to harmful impact on fungus. Seed health testing and identification is first line approach in overseeing seed conceived sicknesses of plant. Salicylic acid utilized as a successful and harmless to the ecosystem plant defender as well as plant development controller and they are economic and easily applicable by nursery workers and poor farmers in developing mass planting stock.

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Conflict of interest

I, corresponding author, on behalf of all contributing authors, declare that there is no conflict of interest regarding manuscript and the information in this manuscript is true and complete to the best of my knowledge and belief.

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