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Impact of Plant Growth Regulators on Seed Health during Accelerated Ageing Test in Chickpea [*Cicer arietinum* (L.)] Seeds

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ABSTRACT

Background: Research was conducted to ascertain the impact of hormonal seed strengthening on seed health status under an accelerated ageing test in chickpea *Cicer arietinum* (L.) at the state seed testing laboratory during the *Rabi* seasons of 2018-19 and 2019-20. Four replications of the Blotter test were performed CRD plan. As per continuous evaluation and observation, under accelerated ageing increased pathogens were observed with increased temperature and RH, the seeds treated with salicylic acid resulted with least pathogen infection while compared to other treatments.

Methods: The current experiment was conducted in the state seed testing laboratory, Department of Genetics and Plant Breeding, SHUATS, Prayagraj, Uttar Pradesh, between the years of 2018 and 2020. 37 distinct treatment combinations were employed in the experiment. The seeds were placed in an accelerated ageing condition with high temperature and relative humidity. Hormonal seed strengthening is the activity of infusing PGRs into old seeds to make them fresh again with the help of PGRs (salicylic and giberellic acid) treatments into old seeds and increase the vigour index of seeds.

Result: In this experiment chickpea seeds treated with combination of gibberellic acid and salicylic acid resulted in least infection of seed pathogens we can recommend these for maintaining seed health status.

Key words: Artificial ageing, Blotter test, Chickpea, PGRs invigoration, Seed health.

INTRODUCTION

A miniature plant embryo known as a seed is encased in a seed coat. Gymnosperm and angiosperm plants produce it after their ripened ovules mature. The fundamental component of all food crop production is the seed. In recent years, seeds have developed into a global trade good for the exchange of genetic material. But seeds are also having a possibility to spread plant disease to new areas and seeds act as vector for plant disease transmission from season to season (Walcott *et al.,* 2006). Thus, most nations regularly conduct seed health testing for domestic seed certification, quality evaluation and plant quarantine (FAO, 2010). Modern agricultural research has long acknowledged the importance of seed health for desired plant populations and successful harvests (Rahman *et al*., 2008). Pulse crops play an important role in Indian agriculture and India is the largest producer and consumer of pulses in the world. Pulses contain a high percentage of quality protein nearly three times as much as cereals (Upadhayay *et al*., 2016). Chickpea (*Cicer arietinum* L.) belong to family Fabaceae, is an important and in expensive source of protein in human food and animal feed. After soyabeans, peanuts and peas, Chickpea is the world's fifth most important legume. Chickpea are generally grouped into two types, the desi type with small angular, dark colored and rough seeds, cultivated mostly in the Indian subcontinent, Ethiopia, Mexico and Iran and the kabuli type with large, light colored and smooth seeds, cultivated mainly in Southern Europe, Northern Africa, Chile and Afghanistan (Zohary and Hopf, 2000; Kumar and Dua, 2006).

Chickpea seed (100 g) on an average provides about 5.0 mg 100 g of iron, 4.1 mg 100 g of zinc, 138 mg 100 g of magnesium and 160 mg 100 g of calcium. About 100 g of chickpea seed can meet daily dietary requirements of iron (1.05 mg/day in males and 1.46 mg/day in females) and zinc (4.2 mg/day and 3.0 mg/day) and 200 g can meet that of magnesium (260 mg/day and 220 mg/day).

Delouche (1965) first introduced accelerated ageing as a test for seed quality at the seed technology laboratory, Mississippi State University, USA. Originally, it was developed as a test to estimate the durability of seeds in

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warehouse storage. Subsequent studies have confirmed the accuracy of this test in predicting the lifetime of a variety of different species of seeds within the storage range (Delouche and Baskin, 1973). Artificial ageing treatments take advantage of the fact that seed ageing process is determined by theseed moisture contant and temperature. alterations of these factors, increases the rate of seeds deterioration pattern.

The physiological and biochemical changes during seed ageing have been extensively reviewed (McDonald, 1999; Jatoi *et al*., 2001). Quality improvement of the seeds and enhancement of the yield of the crops (Jeyakumar *et al.,* 2008; Meena *et al.,* 2016). show that priming can enhance enzyme activity in aged seeds (Monajjem *et al.,* 2023). Invigorated seeds indicated that highest dehydrogenase activity (OD 10 min^{-1}), catalase activity, peroxidase activity (OD 10 min^{-1}) (µg H_2O_2 mg^{-1} min^{-1}) under accelerated aged seeds of chickpea (Hridya *et al*., 2018). The aging methods had a significant negative impact on chickpea seeds physicochemical quality. Catalase activity was dramatically reduced in accelerated ageing as a result of greater temperature and relative humidity (Patil *et al*., 2024). Under storage conditions, seed typically lost their viability within a few days or weeks (Murthy and Kumar, 2003).

In agricultural research, seed health is a better identical factor for desired plant population and high yield (Rahman *et al.,* 2008). Seedborne infections are a major issue and they may even be responsible for the re-emergence of old diseases as well as the spread of diseases to new areas (Gitaitis and Walcott, 2007). Seedborne infections pose a significant risk to seedling production (Walcott, 2003). Seed is now responsible for the transmission of plant pathogens across huge distances, natural obstacles and political borders more than ever before (Gitaitis and Walcott, 2007). Seed-borne fungus are one of the most significant biotic constraints in seed production in the world. They cause both pre- and post-emergence grain death, influence seedling vigour and consequently cause some reduction in germination as well as variation in plant shape (Van Du *et al*., 2001; Rajput *et al*., 2005; Niaz and Dawar, 2009). Seed-borne pathogens can cause germination loss, discoloration and shrivelling, the spread of plant diseases, the introduction of new strains or physiologic races of the pathogen along with new germplasm from other countries and the production of toxin in infected seed (Agarwal and Gaur, Undated). Fungi dominate all other species of plant pathogens and have an important impact on agricultural production due to their capacity to cause diseases overall cultivated crops, resulting in yield losses (Paplomatas, 2006). Keeping in view above facts, the present investigation research experiment has been planned with the objective impact of treatment with hormonal seeds on the health status of Chickpea seeds after accelerated aging.

MATERIALS AND METHODS

In the accelerated Ageing method procedure each treatment fresh chick variety seeds were tied three times in a row in a fine muslin linen bag. On wire mesh desiccators, the tied seeds were put. Water filled the lowest portion of the desiccators (Patil *et al.,* 2019). To maintain the pH of the water, table salt was added and was tied in a fine muslin cloth bag. The tied seeds were placed in desiccators on a wire mesh. The lower part of the desiccators were filled with water. There would not be any direct contact between water and the seed. The jar was covered with thelid and sealed with paraffin wax to make it air tight. The jar was then placed in the accelerated ageing chamber maintained at 35°C and 45°C temperature and 90%, 100% relative humidity for different variation and duration of treatment effect used 0 (control), 2, 4 and 8 days. The jar was removed after this period and the seeds were cooled in adesiccator. The seeds were then tested for normal germination test (Anonymous, 2004). For the preparation of solution of the growth hormone, 100mg. of each chemical were taken in a beaker. These chemicals were added in 1000 ml. of distilled water with constant stirring. The volume of solution was finally constitutes to one litter and then it became 100 ppm stock solution of each chemical. The flasks containing chemicals were covered with muslin cloth to avoid any contamination. After preparation of solution of gibberellic acid and salicylic acid, chickpea seeds were soaking in respective solution for 12 hour at 25°C temperature. After 12 hr of soaking the solution were drained out from the beaker and soaked seeds were air dried to original weight and then placed for germination in laboratory under controlled condition.

Seed health (blotter method)

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 under aseptic condition and incubated at 20±2°C for 7 days with alternate cycles of 12 h in near ultraviolet light (NUV) range and 12 h in dark conditions. On the eighth day, the seeds were examined for the presence of fungal infection (Plate 1-2). The number of infected seeds were counted and the mean value was expressed in percentage (ISTA, 2010).

The environment has an impact on how the pathogen manifests itself during incubation. The blotter test shows seed infection as indicated by the development of mycelium and fruiting bodies, as well as germinated seedling infection as shown by symptoms in young plants in some research. In seed wellbeing testing for seed-borne contagious microorganisms the blotting surface test is no uncertainty perhaps the most significant strategy accessible (Limonard, 1966). Blotting surface tests were similar to germination experiments in that seeds were palced on damp layers of blotting surface paper, where they were then hatched under circumstances that promote parasitic development. At that time, the seed may be allowed to grow and parasite seed-borne infections might appear as any appropriate indications or symptoms (Plate 3). The natural conditions during incubation have an effect on

the microorganism's indications. The blotting surface test indicates seed contamination, as evidenced by the detection of mycelium and fruiting bodies and, in a few instances, contamination of set seedlings, as evidenced by suggests on young plants.

The most popular tool used for identifying seed borne fungi is the Agar plate (Rao and Bramel, 2000). Incubation methods enable the identification of viable fungal material even at the early stages of fungal growth. To assist the growth of seed-borne fungus, seeds are frequently placed on sterile agar media (most commonly potato dextrose or malt agars). Agar plate methods can be used to quantify the fungal burden, such as seed CFU/gm (dilution plate methods), or to determine the species composition qualitatively (direct plate method) (Plate 4). The simplest approach for measuring the volume of fungal tissue in kernels is the dilution plate method. There are two versions of this technique: the more prevalent pour plate approach and the low contamination of the sample spread plate method. The direct plating approach, on the other hand, is one of the best ways for detecting the composition of grain fungus in terms of genera and species. In this procedure, after disinfecting the surface of the kernels, whole kernels are placed on the surface of the culture medium. As a very efficient method for assessing the internal colonization of kernels by fungi, the direct plating technique can be recommended and is therefore a very helpful tool for evaluating the quality of bulk grain. There are major differences in the use of the agar test, mainly with respect to sample preparation, media selection and incubation temperature and length. Acidic agar with acid Bacterial colonies also form Inhibiting fungal growth on the agar and rendering identification difficult. To decrease bacterial pollutants, acidic agar media may be used to (Trojanowska, 1991). Bacterial colonies often grow on the agar and inhibit fungal growth, making it difficult to identify. This can be avoided by adding an antibiotic, such as streptomycin, to the autoclaved agar medium after it has cooled to 50-55°C (Rao and Bramel, 2000).

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 1% level and 5% level of significance under study.

RESULTS AND DISCUSSION

The experiment consisted of combination of 36 treatments with the untreated control (T_0) of all the seed samples tested, samples from the four replication. The hormonal treatment with Gibberellic acid at RH (90%) and temperature (35^0C) for 2 days ageing showed minimum fungal infection, whereas fungal infection lowers gradually with the increase in relative humidity, temperature and duration of accelerated aging.

Throughout the experiment, the fungal infection varied significantly among the treatments. The lower fungal



Plate 1: Some hard seeds, fungal infection and dead seeds during seed health testing.



Plate 2: During incubation, the manifestations of the pathogen are affected by the environmental.

infection was recorded in seeds treated with T_{13} [Gibberellic acid +RH (90%) +Temp. (35°C) +Days (2)] (0.50), (0.52), (0.51 %) followed by T_{25} [Salicylic Acid +RH (90%) +Temp. (35°C) +Days (2)] (0.54), (0.56), (0.55%), which were on par with T_{28} [Salicylic Acid +RH (90%) +Temp. (40°C) +Days (2)] (0.99%) and T_{19} [Gibberellic acid +RH (100%) +Temp. (35°C) +Days (2)] (0.81 %), while significantly highest was recorded in T_{12} [RH (100%) + Temp. (40°C) + Days (8)] (3.10%). It was seen that there is significant variation among treatments with reason of salicylic acid inhibits the catalase activity and gibberellic aic stimulates the bio synthesis of seed germination. GA_3 had a signal suppressive influence

Table 1: After accelerated aging in chickpea (*Cicer arietinum* L.), hormonal seed treatment had a negative effect on seed health and a positive effect when treated with hormones.

T. no.	Treatments	1st Year (2018-19)	2nd Year (2019-20)	Pooled data (1st and 2nd year)
T_0	Control (Untreated)	1.50	1.55	1.52
T_1	RH (90%) + Temp. (35°C) + Days (2)	2.00	2.06	2.04
T_2	RH (90%) + Temp. (35°C) + Days (4)	2.25	2.32	2.29
T_3	RH (90%) + Temp. (35°C) + Days (8)	2.57	2.65	2.62
T_4	RH (90%) + Temp. (40°C) + Days (2)	2.75	2.83	2.78
T_5	RH (90%) + Temp. (40°C) + Days (4)	2.74	2.82	2.78
T_6	RH (90%) + Temp. (40°C) + Days (8)	2.78	2.86	2.82
T_7	RH (100%) + Temp. (35°C) + Days (2)	2.85	2.94	2.91
T_8	RH (100%) + Temp. (35°C) + Days (4)	2.89	2.98	2.96
T_9	RH (100%) + Temp. (35°C) + Days (8)	2.93	3.02	3.00
T_{10}	RH (100%) + Temp. (40°C) + Days (2)	2.99	3.08	3.02
T_{11}	RH (100%) + Temp. (40°C) + Days (4)	2.96	3.05	3.03
T_{12}	RH (100%) + Temp. (40°C) + Days (8)	3.05	3.14	3.10
T_{13}	Gibberellic acid + RH (90%) + Temp. (35°C) +Days (2)	0.50	0.52	0.51
T_{14}	Gibberellic acid + RH (90%) + Temp. (35°C) + Days (4)	0.69	0.71	0.71
T_{15}	Gibberellic acid + RH (90%) + Temp. (35°C) + Days (8)	1.01	1.04	1.03
T_{16}	Gibberellic acid + RH (90%) + Temp. (40°C) + Days (2)	1.09	1.12	1.11
T_{17}	Gibberellic acid + RH (90%) + Temp. (40°C) + Days (4)	1.04	1.07	1.05
T_{18}	Gibberellic acid + RH (90%) + Temp. (40°C) +Days (8)	1.25	1.29	1.27
T_{19}	Gibberellic acid + RH (100%) + Temp. (35°C) + Days (2)	0.80	0.82	0.81
T_{20}	Gibberellic acid + RH (100%) + Temp. (35°C) + Days (4)	0.75	0.77	0.76
T_{21}	Gibberellic acid + RH (100%) + Temp. (35°C) + Days (8)	1.52	1.57	1.54
T_{22}	Gibberellic acid + RH (100%) + Temp. (40°C) + Days (2)	1.20	1.24	1.22
T_{23}	Gibberellic acid + RH (100%) + Temp. (40°C) + Days (4)	1.75	1.80	1.78
T_{24}	Gibberellic acid + RH (100%) + Temp. (40°C) + Days (8)	2.12	2.18	2.15
T_{25}	Salicylic acid + RH (90%) + Temp. (35°C) + Days (2)	0.54	0.56	0.55
T_{26}	Salicylic acid + RH (90%) + Temp. (35°C) + Days (4)	1.00	1.03	1.01
T_{27}	Salicylic acid + RH (90%) + Temp. (35°C) + Days (8)	0.64	0.66	0.66
T_{28}	Salicylic acid + RH (90%) + Temp. (40°C) + Days (2)	0.97	1.00	0.99
T_{29}	Salicylic acid + RH (90%) + Temp. (40°C) + Days (4)	1.33	1.37	1.34
T_{30}	Salicylic acid + RH (90%) + Temp. (40°C) + Days (8)	1.28	1.32	1.31
T_{31}	Salicylic acid + RH (100%) + Temp. (35°C) + Days (2)	1.65	1.70	1.67
T_{32}	Salicylic acid + RH (100%) + Temp. (35°C) + Days (4)	1.80	1.85	1.84
T_{33}	Salicylic acid + RH (100%) + Temp. (35°C) + Days (8)	1.11	1.14	1.13
T_{34}	Salicylic acid + RH (100%) + Temp. (40°C) + Days (2)	1.74	1.79	1.77
T_{35}	Salicylic acid + RH (100%) + Temp. (40°C) + Days (4)	1.47	1.51	1.50
T_{36}	Salicylic acid + RH (100%) + Temp. (40°C) + Days (8)	1.84	1.90	1.87
	Grand mean	3.05	3.14	3.10
	CD (5%)	0.06	0.05	0.04
	CV	2.2	2.5	2.2
	SE(d)	0.02	0.02	0.02

Where, P1= GA3; P2= SA; H1= 90%; Relative humidity; H2=100% Relative humidity; T_1= 35°C; T_2= 40°C Temperature and D1= 2 days; D2= 4 days; D3= 8 days.

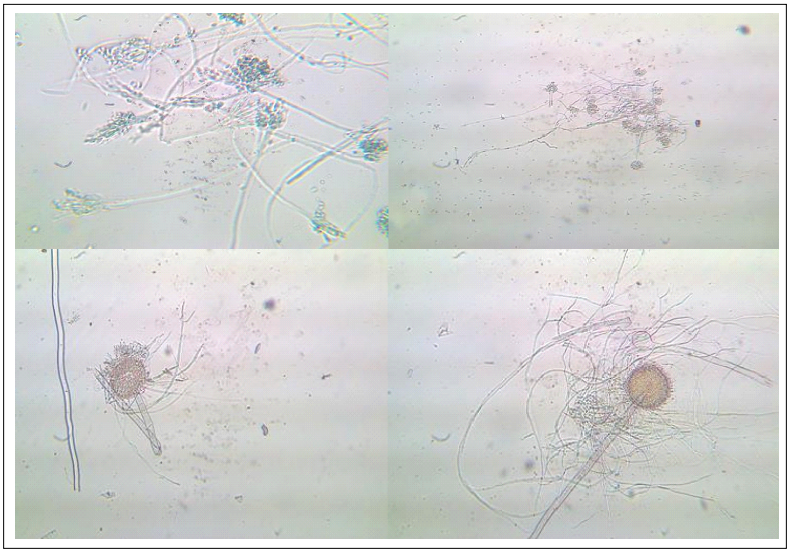


Plate 3: Symptoms of a fungus infection during testing artificially aged.

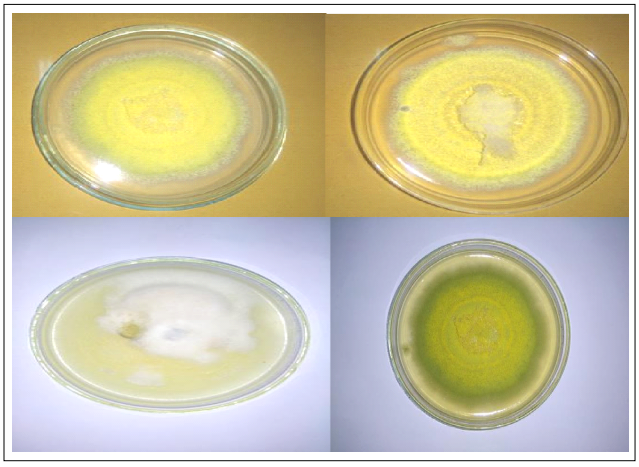


Plate 4: Fungi associated with various treatments of chickpea seeds during accelerated ageing.

on the spore germination of pathogens and mild suppressive effect on spore, salicylic acid germination and growth rate of colony (Degani *et al*., 2015). Emerging evidence indicates that components of GA signaling playing a significant role in the tolerance and vulnerability of plant diseases (Robert-Seilaniantz *et al*., 2011). The Anova that the calculated values of F due to the effects of hormonal treatments after accelerated aging were higher than the respective table values at 1% and 5%, so null hypothesis was rejected. Hormonal seed treatment had a detrimental effect on seed health after chickpea (*Cicer arietinum* L.) accelerated ageing. However, when treated at varying temperatures and humidity levels and then treated with hormone, there was a good positive effect, as shown in Table 1.

CONCLUSION

From the results it is concluded in the present study T_1 to T_{12} is untreated with experimental factors that compare with T_{13} to T_{24} (treated with Gibberellic acid) and T_{25} to T_{36} (treated with Salicylic acid). According to result, the pathogen growth inhibit more in T_{25} to T_{36} treatement experiment due to reason of inhibition of catalase activity of the pathogen by Salicylic acid which stops the cytochrome respiration in the organism and increases the hydrogen peroxide which enhanses the seed germination. In the T_{13} to T_{24} treated experiment T_{13} give the most effective responses at RH 90%, temperature 35°C and in 2 days because the GA for cell proliferation and high RH maintain the moister content of seeds at 35°C to stop the seed deterioration. The Gibberellic acid and salicylic acid has many effects, such as stimulating the plant to form protein-related with pathogenesis and increasing the flowering period, inhibits the formation of ethylene, germinating the seeds and closing wounds. SA was able to significantly reduce *F. oxysporum* growth, because SA is a natural phenolic compound that have inhibitory effect on microbial and that the reason to toxic effect on fungus.

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Conflict of interest

The all authors are declared that they no conflict of interest regarding publication of this article.

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