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APPLICATION OF MANNITOL ON CHICKPEA (*CICER ARIETINUM* L.) ON GERMINATION, VEGETATIVE GROWTH PARAMETERS, AND THEIR RESISTANCE AGAINST *FUSARIUM OXYSPORUM*

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ABSTRACT

In the current investigation, varying concentrations of mannitol (1%, 2%, 3%, 4% and 5%) were administered to chickpeas to assess their impact on germination, vegetative growth parameters, and resistance to *Fusarium oxysporum*. Chickpea seeds treated with mannitol exhibited improvements in both seed germination and seedling vigor. Notably, a 2% mannitol solution demonstrated the most significant growth enhancement in 14-day-old seedlings, with a germination rate of 94% and a vigor index of 3,313.71. Following closely was the 1% mannitol solution, which exhibited a germination rate of 92% and a vigor index of 2,861.50. The vegetative growth parameters revealed heightened levels of chlorophyll, dry weight, fresh weight, relative water content (RWC), and leaf count in the 2% mannitol solution, followed by the 1% solution. Additionally, a noteworthy observation was the reduced incidence of disease in the 2% mannitol solution, indicating enhanced resistance against *Fusarium oxysporum*.

Key words : Chickpea, Chlorophyll, Mannitol, Resistance, *Fusarium oxysporum*, Stress.

Introduction

Mannitol, a naturally occurring 6-carbon sugar alcohol, holds significant importance in pharmaceuticals and food industries, serving as a sweetener (Chen *et al*., 2020). Due to its lack of a glycemic index, there is a high demand for mannitol production. Its application in plants has demonstrated responses to biotic stress and increased tolerance to salinity, making it a crucial player against both biotic and abiotic stressors (Stoop *et al*., 1996).

A substantial portion, approximately 90%, of the world's chickpeas can thrive in arid and semi-arid regions (Mafakheri *et al*., 2010). Chickpeas, being annual legumes, play a pivotal role as a protein source for humans in semi-arid North Africa (Mhadhbi *et al*., 2007). Biotic stresses such as fungal, bacterial, and viral diseases, along with abiotic stresses like insect pests, nematodes and parasitic weeds, significantly impact global chickpea production (Rubiales, 2012).

Plants face various abiotic and biotic stressors that negatively influence their growth, development and productivity. Drought, a prominent stress factor, contributes to the decline in plant growth and productivity on a global scale (Najafi *et al*., 2010). Seed vigour encompasses the properties determining the potential level of activity and emergence of seedlings, involving aspects such as the rate and uniformity of seed germination and seedling growth (Finch *et al*., 2016). To assess plant biomass, it is essential to calculate both wet and dry weights. Data obtained from plant biomass can provide insights into the total number of plants capable of absorbing pollutants from a designated pollution zone (Tangahu *et al*., 2010). Chlorophyll, a primary photosynthetic pigment,

enables plants to absorb light energy from the sun, influencing the colour appearance of leaves and serving as a parameter for crop maturity, quality and freshness. Photometric determination of chlorophyll content involves pigment extraction using an organic solvent (Limantara *et al*., 2014).

Fusarium wilt, caused by *Fusarium oxysporum*, significantly affects most chickpea-growing areas worldwide. Annually, *Fusarium* wilt results in a 10-50% reduction in chickpea yield, attributed to the virulence of the pathogen race in soil and the susceptibility of chickpea cultivars (Juan *et al*., 2000).

Materials and Methods

Collection of seed samples

Infected seeds, leaves and stems showing typical leaf spot symptoms were collected from different chickpea-growing regions of Mandya district, for the isolation of pathogen *Fusarium oxysporum*. The seed samples were collected from farmers, public seed agencies and private sectors.

Screening, isolation and identification of *Fusarium oxysporum*

Isolation, identification and maintenance of pathogen. The diseased plants were analysed in the laboratory and pathogen isolation was subsequently performed there as well. Isolation of the pathogen was carried out by cutting the fragments, at the border of diseased stem. The fragments were surface sterilized with 3% NaOCl for 3 minutes followed by washing with sterile distilled water and transferred on Potato dextrose agar media (PDA) plates. The plates were incubated at 25°C at room temperature for seven days in an inverted position followed by the standard procedures (Machado *et al*., 2004; Mathur and Kongsdal, 2003). The pathogen was purified by regular transferring of the active growth zone of the mycelia into fresh PDA plates, which were stored at 4°C as stock cultures for further studies. The isolation, identification, and confirmation of *Fusarium oxysporum* is based on the examination under different magnifications of a stereomicroscope based on cultural and morphological characteristics (Burgess, 1981).

Preparation of mannitol solution

Preparation of Mannitol solution in different Concentrations as Inducer Treatment 18.217gm of Mannitol was dissolved in a minimum quantity of distilled water until no granules were left over and the final volume was made up to 100 ml using a standard flask. From this stock solution different concentrations of 1%, 2%, 3%, 4% and 5% Mannitol were prepared.

Growth condition

Mannitol-treated seeds were sowed in tray pots with different concentrations (1, 2,3,4,5 and control) for 14 days up to seedlings. The experiment was setup in 3 replications with 6 treatments, mannitol treated seeds were germinated in the paper towel method and the growth parameters were estimated after 14 days.

Effect of Mannitol on seed germination and vigour index

Effect of chickpea seeds Priming with Mannitol on Seed Germination and Seedling Vigour Seeds of chickpea susceptible plants were taken; surface sterilized in 1% sodium hypochlorite solution for 1 min and soaked in different concentrations of Mannitol (100 ml) for 3h and 6h, respectively. Distilled water-treated seeds served as control. The experiment was conducted with 100 seeds in four replicates and repeated thrice. The treated seeds (one set inoculated with the conidia of *F. oxysporum* at $1 \times 10^4 ml^{-1}$ concentration and another set without inoculation) were placed on wetted paper towels (ISTA, 2005) at equal distances and the same was incubated for 10 days at 25 ± 20^0C. Germination percentage was analysed by the method of Abdul-Baki and Anderson (1953). The percentage of germination, root length, and shoot length were recorded and the vigour index was calculated as mentioned below:

$$\text{Percentage of germination} = \frac{\text{No. of seed germinated}}{\text{Total no. of seed plated}} \times 100$$

Vigour index = Seed Germination % × (Mean root length + Mean shoot length).

The concentration of the Mannitol, which showed effective seed germination and vigour Index, was used for further studies.

Determination of vegetative growth parameter

After 14 days, chickpea plants were harvested. Shoot and root length were recorded in centimetres of each plant. This shoot and root dry weight were recorded in grams. Mannitol-treated and controlled seeds were germinated on germination paper and germination percentage is calculated for every concentration (Yadav and Parihar, 2010).

Determination of fresh and dry weight

Five seedlings were selected from the control as well as the experimental setup. The fresh weights were weighed with the help of weighing balance. The average value for each treatment was recorded. The seedlings were then kept in a hot air oven at 70^0C for 24 hrs to obtain a constant weight and dry weight was calculated

(Femina *et al*., 2012).

Determination of the number of leaves

Measurements regarding leaf production and senescence were the total number of nodes on the main stem, the number of nodes on the main stem with senescence, the total plant leaf number (green + senescence) and the number of primary, secondary and tertiary branches. A leaf was counted when its leaflets were unfolded and a green leaf was considered with >50% green area. The number of fallen leaves was counted based on visible leaf scars (Soltani *et al*., 2006).

Determination of Chlorophyll content

100mg of leaf tissue was suspended in 10 ml of 80% acetone mixed well. The supernatant was withdrawn after centrifugation (5000rpm) for 10min. The optical density was measured at 663nm and 645nm in a spectrophotometer. The chlorophyll content was calculated by (Arnon, 1949).

$$\text{Chlorophyll 'a' (mg/f.wt)} = \frac{12.7(\text{OD of 663nm}) - 2.69(\text{OD of 645nm}) \times V}{1000 \times W}$$

$$\text{Chlorophyll 'b' (mg/f.wt)} = \frac{22.9(\text{OD of 645nm}) - 4.68(\text{OD of 663nm}) \times V}{1000 \times W}$$

V = volume of sample

W = weight of fresh tissue

Effect of mannitol for disease incidence

The cultivars of chickpea were collected during the field survey and screened for wilt disease incidence under greenhouse conditions. The collected seed samples were sown in tray pots containing 2:1:1 red soil, sand, and farm yard manure, previously autoclaved. The pots were maintained under greenhouse conditions (25± 2^0C). The 30-day-old seedlings were challenge inoculated. With a spore suspension of *F. oxysporum* (1×10^7 spores mL^{-1}). Each experiment consisted of 50 seeds in a pot 2 seeds each and the experiment was repeated with four replicates. Plants were observed daily for the typical symptoms of wilt disease. At the end of 30 days after challenge inoculation, disease incidence was recorded using the formula (Halila and Strange, 1996).

$$\text{Percent Disease Incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Results

Various parameters were investigated to understand the physiological effects and interactions resulting from the application of different concentrations of mannitol. The findings revealed that the chickpea plants exhibited improved growth, an augmentation in growth parameters, and a reduction in disease incidence, when subjected to a 2% mannitol solution. This was notably observed when compared to other concentrations as well as the control group.

Effect of seed priming with mannitol on seed germination and vigour index of chickpea

Seeds subjected to Mannitol priming at various concentrations were evaluated for their impact on seed germination and seedling vigour after fourteen days. Notably, susceptible chickpea seeds treated with a 2% Mannitol concentration displayed a notable enhancement in seed germination at 94% and seedling vigour at 3,313, surpassing the outcomes observed in all other Mannitol treatments (Table 1). In contrast, untreated control seeds exhibited a seed germination rate of 80% and a seedling vigour of 2,072.

Table 1 : Effect of Mannitol on chickpea seed germination and seedling vigour after seed treatment.

Concentration	% of Germination	Seedling vigor
Control	80.00±0.23	2072±16.15
1%	92.50±0.14	2861±06.50
2%	94.00±0.12	3,313±12.48
3%	86.00±0.24	3193.9±19.65
4%	87.25±0.16	2990±14.56
5%	84.50±0.05	2261±05.25

Evaluation of seed treated with Mannitol on vegetative growth parameters of chickpea under greenhouse conditions

Chickpea seeds subjected to mannitol priming, in addition to a control group, were assessed for their impact on vegetative growth parameters, including plant height, wet weight, dry weight, number of leaves, and chlorophyll content after the soaking period. The study results revealed a significant ($p \leq 0.05$) enhancement in growth parameters compared to the control plants (Table 2). Specifically, chickpea plants primed with a 2% concentration of mannitol exhibited the most substantial improvements in plant height (17.053), shoot fresh weight (2.99 g $plant^{-1}$), shoot dry weight (12.58 g $plant^{-1}$) and the number of leaves (250 $plant^{-1}$). This was followed by plants treated with 3% and 4% concentrations. Correspondingly, the chlorophyll results mirrored the findings of the growth parameter studies. The highest chlorophyll content, specifically 0.18351 mg g^{-1} in chlorophyll-a, was observed in chickpea plants treated with a 2% concentration of mannitol. Similarly, an increased chlorophyll content of 0.27175 mg g^{-1} in chlorophyll b was noted. These results align with the

Table 2 : Effect of mannitolon vegetative growth parameters of Chickpea under greenhouse conditions.

Concentration	Plant height in cm (average)	Fresh weight in grams	Dry weight in grams	No. of leaves	Chlorophyll in mg	
					a	b
Control	11.06 ±0.38	0.91 ± 0.06	0.72 ± 0.03	138.50 ± 4.55	0.008 ± 0.0003	0.009 ± 0.000
1%	14.85 ±0.25	1.50 ± 0.17	1.29 ± 0.15	212.25 ± 3.81	0.004± 0.0003	0.006 ± 0.0000
2%	17.63 ± 0.23	2.99 ± 0.11	12.58 ± 0.05	250.25 ± 4.55	0.183± 0.0008	0.271 ± 0.0002
3%	16.55 ± 0.19	2.33 ± 0.09	11.09 ± 0.05	246.50 ± 5.54	0.182± 0.0012	0.208 ± 0.0001
4%	15.38 ± 0.15	2.22 ± 0.10	10.93 ± 0.08	133.75 ± 3.72	0.141 ± 0.0005	0.125 ± 0.0000
5%	14.14 ± 0.24	1.04 ± 0.02	0.95 ± 0.04	88.75 ± 3.56	0.013± 0.0001	0.022 ± 0.0000

Table 3 : Greenhouse experiment showing incidence of *Fusarium oxysporum* in Mannitol primed Chickpea seeds.

Concentration	% of disease incidence
Control	40.00±0.50
1%	34.50±0.48
2%	14.00±0.36
3%	18.00±0.12
4%	21.25±0.28
5%	31.50±0.40

observed maximum seed germination, vigour and vegetative growth parameters, as well as reduced disease incidence, in seeds treated with 2% mannitol.

Evaluation of seed treated with mannitol for disease incidence in chick pea against *Fusarium oxysporum*

Chickpea plants treated with mannitol were subjected to evaluation for disease incidence, specifically infected with *Fusarium* wilt caused by *Fusarium oxysporum*. The results revealed a significant reduction in disease incidence in seedlings treated with 2% mannitol, followed closely by those treated with 3%, in stark comparison to the control group (Table 3).

Discussion

Numerous studies have explored the use of chemicals to activate latent defence mechanisms in plants when facing pathogenic infections. Mannitol, in particular, plays a pivotal role in signal transduction pathways leading to systemic acquired resistance in plants. Seeds primed with Mannitol have demonstrated higher yields compared to control plants. Monaim *et al*. (2012) reported significant growth-promoting responses in tomato seedlings sprayed with Mannitol, resulting in effective control of wilt disease in tomato plants. Additionally, Mannitol is crucial in pathogenesis, maintaining a balance in cell reinforcements produced by both plants and animals (Meena *et al*., 2015).

The pre-treatment of seeds with a mannitol solution has been linked to increased biomass production in plants. This enhancement in biomass may be attributed to the utilization of mannitol in leaves, acting as a source of carbon and nitrogen. Changes in concentration affected plant growth characteristics such as total leaf area, leaf number and total fresh and dry mass. Khalid *et al*. (2011) noted a decrease in these parameters in mannitol-untreated plants. In the present study, pre-treating seeds with a 2% mannitol solution significantly enhanced leaf number, wet weight, and dry weight in chickpea plants, positively correlating with increased plant biomass.

High biomass production is generally associated with the leaf photosynthetic rate, which depends on factors such as stomatal conductance and the quality of leaf photosynthetic pigments like chlorophyll a and b. Similar effects were observed in marigold plants by Afzal *et al*. (2011), where priming with 2% and 4% mannitol significantly increased germination parameters and shoot and root lengths compared to other pre-sowing seed treatments, including the control group. Notably, disease incidence decreased in chickpea seedlings treated with 2% Mannitol.

Physiological and biochemical analyses suggest that species metabolizing mannitol have advantages over those exclusively translocating sugars. Mannitol's role as a 'compatible solute' contributes to increased tolerance to salt and osmotic stress. Moreover, mannitol may play a crucial role in plant responses to pathogen attacks (Johan *et al*., 1996). In this study, mannitol-treated seedlings, particularly those treated with 2%, exhibited lower disease incidence compared to other concentrations. These findings align with Rokozeno *et al*. (2020) research, where mannitol-treated seedlings showed lower disease incidence percentages than other treatment combinations. Consequently, the application of a 2% mannitol solution has proven to be effective in promoting proper plant growth.

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