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IMPACT OF SEED PRIMING ON GROWTH AND YIELD RELATED TRAITS OF BLACK CUMIN

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ABSTRACT

A randomized block design with three replications utilizing different treatment combinations was used in study conducted at the Horticultural Research Station, Mandouri farm, during 2019–23 *rabi* season, to know the impact of seed priming on various traits in black cumin. Among them, T_8 (seed treatment with *Trichoderma viride* @ 2g/lit) recorded highest plant height of 28.15cm at first flowering , 41.25cm at 50% flowering and 80.71 cm at harvest followed by T_4 (seed treatment with Salicylic acid @200ppm + Carbendazim spray@ 2g/lit at 30DAP) with (25.41cm, 37.06 cm and 76.08cm, respectively). Primary and secondary branches were higher in T_8 with (6.66 and 27.30, respectively). T_4 recorded highest number of capsules (27.36), capsule size (1.33cm), dry capsule weight (1.44g), number of seeds per plant (2045.75), 1000 seed weight (1.95g), straw yield (15.11g), fresh plant weight (37.68g), dry plant weight (21.44g), seed weight per plant (4.16g), seed yield per plot (0.83kg), projected seed yield (8.32 q ha^{-1}) and BCR of 1:1.92. Projected oil yield was more for treatment T_8 with 4.59 L ha^{-1}followed by T_4 (4.19 L ha^{-1}) against control plots (T_9). Salicylic acid and Trichoderma were crucial in regulating physiological and biochemical processes, may have contributed to the yield enhancement.
***Keywords*: branches, capsules, growth, salicylic acid, seed priming, Trichoderma and yield**

Introduction

The use of herbal remedies has grown in the modern day. Numerous factors contribute to this rise in usage, but the main one is the general perception that these herbal products are safe and natural (Kazemi, 2014). Among the seed spices cultivated, black cumin, also known as nigella, is regarded as a miracle spice with significant therapeutic benefits in addition to its inherent flavor (Naz, 2011). The Prophet Muhammad's (SAW) famous quote, "Hold on to use of the black cumin seed, it has a remedy for every illness except death", is another reason for its fame (Bukhari, 1985). Black cumin is a nutrient-dense herb that contains monosaccharides (Heinrich *et al*., 2004). According to scientific studies, its composition *i.e.,* the amounts of moisture, oil, proteins, ash, and total carbohydrates ranges from 3.80 to 7.00%, 22.00 to 40.35%, 20.85 to 31.20%, 3.70 to 4.70%, and 24.90 to 40.00%, respectively (Atta, 2003); (Takruri and Dameh, 1998). The seed contains 30% fixed oil and 0.3 to 0.4% essential oil (Hala *et al*., 2016). Plant sterols, flavonoids, dietary fibers, antioxidants, vitamins, and η-3-fatty acids are examples of phytochemicals that help people stay healthy (Razavi and Hosseinzadeh, 2019) and lower their chance of developing a number of diseases (Ramaa *et al*., 2006; Manach *et al*., 2005). Seeds are used both as culinary and liquor preparation (Luetijohann, 1998) whereas its essential oil solely contains volatiles, black cumin fixed oil is a lipid component that contains fatty acids, fat-soluble vitamins, and meagre amount of volatiles (Thippeswamy and Naidu, 2005). The volatile oil is distinguished by significant concentrations of p-cymene (14.8%) and trans-anethole (38.3%) as well as phenyl propanoids (Nickavar *et al*., 2003).

In India, not many farmers are growing black cumin crop on commercial basis; crop productivity was reported to be between 300-500 kg ha^{-1}. So, there is a discrepancy between its production and demand, a very small quantity of yield is obtained that is insufficient to meet the national requirements. An increase in external Na+ concentration is detrimental to the influx of K^+ into cells, which is crucial for plant growth. It has a number of detrimental impacts, including slower plant growth and effects on the photosynthetic process (Hameed *et al*., 2021). Because of osmotic pressure, which limits water uptake, or salt and chloride ion toxicity, salinity has a deleterious impact on seedling germination, growth and defensive mechanism (Shahzad *et al*., 2019). Due to the salinity, collar rot disease and a lack of scientific management techniques, the area of black cumin has steadily decreased. The potential for black cumin production in India can therefore be greatly increased. When crops are cultivated in difficult conditions, seed priming is the greatest way to address germination-related issues proposed by Heydecker and Coolbear (1973). It's a pre-sowing procedure where seeds are somehow soaked to a moisture content that prevents radical protrusion but is enough to start the early germination events (imbibition's) (Abd and Hoda, 2016). Under a variety of environmental circumstances, it can improve the percentage rate of germination and seedling emergence, ensuring appropriate stand establishment (Dotto and Silva, 2017). Keeping the importance in view and lack of the above consorted work, the present piece of research was designed to address the several issues related to the performance of the crop in the Gangetic alluvial zones of West Bengal.

Material and Methods

Field experiments were carried out at the Horticultural Research Station, Mandouri farm, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, between 2019 and 2023. The purpose of the study was to ascertain the effects of several seed priming treatments on collar rot disease and seed output of the Ajmer Nigella-1 variety. The study employed a fully randomized block design with three replicates. The main plots' plant densities and the subplots' fertilizer treatments were assigned at random. The dimensions of each experimental unit were 1 × 1.5 meters. For twenty-four hours, the seeds were soaked in water. According to (table 1), chitosan and salicyclic acid were first dissolved in 10 milliliters of ethanol and then makeup to one liter of water. The seeds were then soaked for four hours according to their respective concentrations, and finally they were planted in the field. *Trichoderma* and carbendazim seed treatments are applied directly to soaked seeds before they were planted in the field; the other treatments were carried out as indicated in the table. Seeds were sown for each of the four growing seasons during the first two weeks of November. The mature stage of the plants was collected in the first two weeks of April. At the moment of fruit ripening, data were recorded. The seeds were subjected to hydro-distillation for 3 hours using a Clevenger type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage. Fertilizers were applied in two separate doses (70:50 NP), a base dosage prior to planting and the remainder fertilizer applied 45 days following plant emergence but before blooming. The growth and yield characteristics for which data were recorded included measurements of the plant's height in centimeters at the physiological maturity stage, from ground level to the tip of the plant. To find out how many primary branches each plant has, five tagged plants from the four center rows were counted. Subsequently, the number of seeds per capsule and the number of capsules per plant were counted individually for each plant and capsule, respectively. The thousand seed weight was calculated by counting, weighing, and expressing in grams the seeds of each of the five tagged plants. To calculate the straw yield, the total weight of the plants from the center sub-plot areas was measured after three days of drying and harvesting. In order to calculate the predicted seed production, the seed weight of the plants was measured. The data collected from agricultural attributes were statistically evaluated of variance at a significance level of 5% using the SAS program (SAS, 1989) and the Panse and Sukhatme (1985) method. The Duncan Multiple Range Test was then compared using the average values.

Table 1: Treatment combinations

T_1	Seed treatment with Salicylic acid @ 200ppm
T_2	Seed treatment with Chitosan @ 500ppm
T_3	Seed treatment with Carbendazim @ 2g /kg
T_4	Seed treatment with Salicylic acid @ 200ppm + Carbendazim spray @ 2g/lit at 30DAS
T_5	Seed treatment with Chitosan @ 500ppm + Carbendazim spray @ 2g/lit at 30DAS

T_6	Seed treatment with Salicylic acid @ 200ppm + *Trichoderma viride* @ 2g/lit spray at 30DAS
T_7	Seed treatment with Chitosan @ 500ppm + *Trichodermam viride* @ 2g/lit spray at 30DAS
T_8	Seed treatment with *Trichoderma viride* @ 2g/lit
T_9	Control (water)

Results and Discussion

Priming, a pre-sowing seed treatment, has been utilized to increase yield, stand establishment, germination, and seedling emergence time (Khalid, 2012). Among all treatments (Table 2), T_8 (seed treatment with *Trichoderma viride* @ 2g/lit) recorded highest plant height of 28.15cm at first flowering , 41.25cm at 50% flowering and 80.71 cm at harvest, *Trichoderma* can quickly adsorb to the roots of crops for propagation and the hyphae quickly wrap the roots of crops through its rapid growth and reproduction, it can seize nutrients and space near the plant rhizosphere, consume oxygen in the air, and inhibits the growth of plant pathogenic fungi; cause physiological and metabolic changes and produce a variety of secondary metabolites that act as elicitors (Basinka *et al.*, 2020); (Oszust *et al.*, 2020) and Panchalingam *et al.*, 2022) followed by T_4 (seed treatment with Salicylic acid @ 200ppm + Carbendazim spray@ 2g/lit at 30DAS) with (25.41cm, 37.06cm and 76.08cm, respectively). The use of salicylic acid, a recognized growth regulator that regulates stomatal conductivity, ion uptake by roots, transport photosynthetic rate, membrane permeability and transpiration, may have contributed to the rise in plant height by increasing the number of nodes (Chaudhary and Sidhu, 2022). Lal *et al.* (2013) supports these findings with 46.74cm plant height in black cumin, Kazemi (2014), Kabiri *et al.* (2012), and Nazmul (2017) supports the present findings. According to Miniawy *et al.* (2013) the application of chitosan and salicylic acid improved stem growth, increased internodal distance and girth in a number of crops to (Kabiri *et al.* 2014).

Primary and secondary branches were higher in T_8 with (6.66 and 27.30, respectively). *Trichoderma* not only helps in growth promotion but improves nutrient utilization efficiency, enhances plant resistance, agrochemical pollution environment and successful defense against soil-borne pathogens (Fontana *et al.*, 2021); (Sanchez *et al.*, 2021) and (Tilocca *et al.*, 2020). The active substances produced by Trichoderma are recognized by plants, thus activating the signal transduction pathway and inducing the production of plant system resistance (Tyskiewicz *et al.*, 2022). The microbial determinants recognized by microorganisms are called microbe-associated molecular patterns (MAMPs) (Baazeem *et al.*, 2021) may have prevented collar rot infection in young plants. It may also have been caused by the solubilization and sequestration of numerous plant nutrients, including P, Mn, Fe, and Zn, which contributed to the plants' increased growth (Kim *et al.*, 2009). *Trichoderma* can significantly enhance the Na+ efflux from the root system of and its transport to the upper ground, ensure K+ absorption and maintain the ion balance in the plant, thus reducing the damage of PSII caused by ion toxicity and oxidative stress, protecting photosynthetic pigments, reducing salt stress (Al-surhanee, 2022).

T_4 recorded highest number of capsules (27.36), capsule size (1.33cm), dry capsule weight (1.44g) followed by T_8 with number of capsules (25.73), capsule size (1.30cm), fresh capsule weight (2.47g), dry capsule weight (1.39g) and remaining treatments were on par with T_4. According to the observations, salicylic acid (SA) treatments stimulated the mitotic system of the apical meristem of roots, increasing the level of cell division and improving plant growth (Yadav *et al.*, 2020). This growth-promoting effect promoted the differentiation of more auxillary buds into capsules (Farouk and Osman, 2011).

According to reports, the photosynthetic apparatus is harmed by the oxidative effects of salt and biological stress on a variety of levels, including pigments, stomatal function and gas exchange, thylakoid membrane structure and function, electron transport, and enzymes (Zahra *et al.*, 2022) fluctuates chlorophyll levels in plant development. Numerous studies that revealed either an increase or a decrease in photosynthetic pigments predicted that the administration of SA impacts on chlorophyll which influences directly on yield promoting parameters (Sousa *et al.*, 2022); (Maia *et al.*, 2023). T_4 showed maximum number of seeds per plant (2045.75), 1000 seed weight (1.95g) followed by T_8 with number of seeds per capsule (93.03), 1000 seed weight (1.93g). *Trichoderma* regulates tricarboxylic acid cycle (TAC) and hexose monophosphate pathway (HMP) to promote growth by enhancing succinate dehydrogenase and glucose-6-phosphate dehydrogenase activities (Manganiello *et al.*, 2018). It produces acidic substances that can dissolve insoluble trace elements in soil and provide more nutrition to plants (Samuelian, 2016).

T_4 recorded straw yield (15.11g), fresh plant weight (37.68g), dry plant weight (21.44g) followed by

T_8 with straw yield (14.30g), fresh plant weight (36.53g), dry plant weight (18.55g) against control plots (table 2 & 3). After treatment with *Trichoderma* strain can upregulate the expression of xylanase genes has a significant growth-promoting effect and impacts on biomass of the crop (Karuppiah *et al*., 2019). It can induce the production of pathogenesis-related proteins and other defensive metabolite (Igantenko *et al*., 2019). Salicylic acid positively impacts a plant's capacity for withstanding biotic and abiotic stress as well as defensive responses (Jangra *et al*., 2022) .Nonetheless, the general improvement in vegetative development brought about by both biological and non-biological techniques of controlling fungi (Anshupranay *et al*., 2019) favorably regulated collar rot disease at an early stage, improved crop stand, blooming and ultimately culminated in higher seed yield attributes (Ramadan *et al*., 2013).

Polygenes control the complicated attribute of yield, which is linked to multiple other factors that contribute incrementally (Somayyeh and Ali, 2012). Improvements in plant height, the number of primary and secondary branches, capsule size, weight, and number of seeds per capsule, among other characteristics, are known to be associated with increased yield in nigella with application of salicylic acid greatly enhanced these characteristics, (Elhamahmy *et al.,* 2016) resulted in improved yield qualities in the current study, which could be because of physiological and biochemical processes that are regulated during plant phenology (Hussain and Mallik, 2008). By applying SA had the greatest ameliorating influence on these yield parameters *i.e.,* seed weight per plant (4.16g), seed yield per plot (0.83kg) and projected seed yield (8.32q ha^{-1}) recorded highest in T_4 followed by T_8 with seed weight per plant (3.73g), seed yield per plot (0.74kg) and projected seed yield (7.46q ha^{-1}) (Figure1). SA increases seed weight by reducing ROS (Jahan *et al.,* 2019). The capacity of SA to scavenge ROS depends on concentration, but less so at lower levels (Van Nguyen *et al*., 2022). Lal *et al.* (2013) obtained higher seed yield (2007.83 kg ha^{-1}) in black cumin. High agricultural yields can be achieved by salicylic acid therapy results in high seed production (Hanif *et al.,* 2024), rates of food nutrition, water potential value, which improves plant growth and germination rates in both salt- and stress-free conditions (Kantoudi *et al*., 2022).

Projected oil yield was more for treatment T_8 with (4.59 Lha^{-1}) followed by T_4 with (4.19 Lha^{-1}) (Figure 2). *Trichoderma Sp.* is also known to produce plant growth stimulators and beneficial chemicals such as indoleacetic acid (IAA), glucose oxidase and harzianolide, to promote the development and growth of plant roots by secreting phytase and ferritin to promote the absorption of P and Fe by plants (Lombardi *et al.,*2020), decomposes soil organic matter; increases the supply of soil nutrients; improves crop photosynthetic efficiency, Al-Hazmi and Tariq Javeed (2016) which can boost their vigor and consequently improving agronomic traits and increases production (Amel *et al*., 2010).

With a maximum net return of Rs. 79921.29 per hectare during the study, T_4 (salicylic acid @ 200 ppm + carbendazim spray @ 2g/lit at 30DAP) had the highest Benefit Cost Ratio of 1.92, according to the data on economic analysis shown in Table 4. *Trichoderma viride* seed treatment at 2g/lit had a net return of Rs. 62815.75 per hectare and a BCR of 1.73. Therefore, seed priming with salicylic acid at 200 ppm and carbendazim spray at 2 g/lit at 30DAP can be suggested as a way to increase the crop's economic returns and profitability.

Conclusion

Overall, the research showed that fungicide and PGR pre-sowing treatments improve germination and seed quality metrics while also boosting nigella seed vigor, germinability, protecting from stress by improving plant growth, enhancing morpho-physiological and biochemical attributes inducing defense mechanisms. In light of the findings, it may be concluded that black cumin seeds treated with 200 ppm salicylic acid + carbendazim spray (2 g/lit at 30DAS) and *Trichoderma viride* (2 g/lit) performed the best and should be suggested to farmers in West Bengal's Gangetic areas.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

The Authors D.K. Ghosh and A. Bandyopadhyay conceived and designed the study. Data collection and experimentation done by Roja Ramani under the guidance of D.K.Ghosh. All authors analyzed the data and critically revised the article. All authors contributed to editing and reviewing of the manuscript. All authors approved the manuscript.

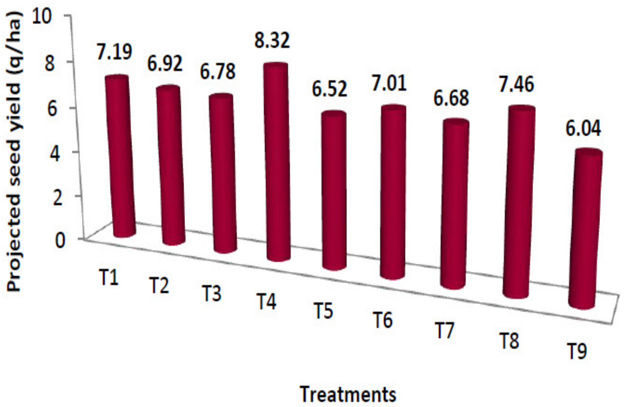

Fig. 1: Impact of seed priming on projected seed yield in black cumin

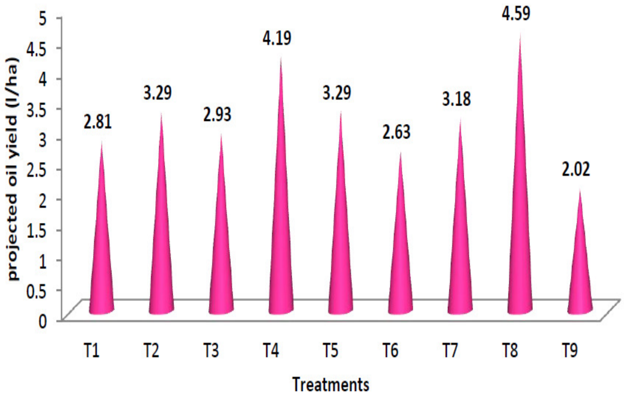

Fig. 2: Effect of seed priming on projected seed yield in black cumin

Table 4: Economic analysis on impact of seed priming in black cumin

Treatments	Yield (q ha^{-1})	Expenditure (Rs. ha^{-1})	Gross Returns (Rs. ha^{-1})	Net Returns (Rs. ha^{-1})	BC ratio
T_1	7.19	86522.28	143800.0	57277.72	1.66
T_2	6.92	87650.28	138400.0	50749.72	1.58
T_3	6.79	86531.38	135733.3	49201.95	1.57
T_4	8.32	86545.38	166466.7	79921.29	1.92
T_5	6.53	87673.38	130533.3	42859.95	1.49
T_6	7.02	86531.58	140333.3	53801.75	1.62
T_7	6.69	87659.58	133733.3	46073.75	1.53
T_8	7.47	86517.58	149333.3	62815.75	1.73
T_9	6.04	86508.28	120800.0	34291.72	1.40

Table 2: Impact of seed priming on growth related traits of black cumin.

Treatment	Plant height at first Flowering (cm)	Plant height at 50% flowering (cm)	Plant height at Harvest (cm)	Number of Primary branches	Number of Secondary branches	Number of Capsules per plant	Capsule size (cm)	Fresh Capsule Weight (g)	Dry Capsule weight (g)
T_1	23.00^{bc}	34.64^{bc}	64.46^{cd}	5.36^{bc}	21.13^{b}	14.16^{e}	1.06^{cd}	1.85^{d}	1.37^{a}
T_2	21.95^{c}	34.85^{bc}	65.90^{cd}	4.70^{c}	17.98^{bc}	21.13^{cd}	1.15^{bc}	2.17^{bcd}	1.23^{ab}
T_3	23.51^{bc}	33.80^{bc}	66.16^{cd}	5.86^{ab}	19.43^{bc}	21.88^{bcd}	1.23^{ab}	2.54^{a}	1.38^{a}
T_4	25.41^{ab}	37.06^{b}	76.08^{ab}	6.21^{ab}	21.96^{b}	27.36^{a}	1.33^{a}	2.01^{cd}	1.44^{a}
T_5	22.20^{bc}	33.85^{bc}	68.93^{c}	5.56^{bc}	19.41^{bc}	22.16^{bc}	1.15^{bc}	1.91^{cd}	1.27^{ab}
T_6	23.71^{bc}	33.98^{bc}	71.46^{bc}	5.80^{b}	20.36^{b}	18.01^{de}	1.24^{ab}	2.38^{ab}	1.36^{a}
T_7	22.23^{bc}	32.80^{c}	68.71^{c}	5.50^{bc}	21.66^{b}	21.23^{cd}	1.21^{ab}	2.25^{abc}	1.37^{a}
T_8	28.15^{a}	41.25^{a}	80.71^{a}	6.66^{a}	27.30^{a}	25.73^{ab}	1.30^{a}	2.47^{ab}	1.39^{a}
T_9	17.91^{d}	27.67^{d}	60.66^{d}	4.73^{c}	14.88^{c}	16.33^{e}	0.96^{d}	1.97^{cd}	1.04^{b}
S. Em (±)	1.01	1.05	2.15	0.26	1.56	1.24	0.03	0.11	0.07
C.D (P=0.05)	3.07	3.17	6.51	0.79	4.72	3.77	0.11	0.33	0.22

[T_1-Seed treatment with Salicylic acid @200ppm, T_2- Seed treatment with Chitosan @500ppm,T_3-Seed treatment with Carbendazim @2g /kg, T_4- Seed treatment with Salicylic acid @200ppm + Carbendazim spray@ 2g/lit at 30DAP,T_5- Seed treatment with Chitosan @500ppm + Carbendazim spray@ 2g/lit at 30DAP, T_6-Seed treatment with Salicylic acid @200ppm + *Trichoderma viridae* @ 2g/lit spray at 30DAP, T_7-Seed treatment with Chitosan @500ppm + *Trichoderma viridae* @ 2g/lit spray at 30DAP, T_8-Seed treatment with *Trichoderma viridae*@ 2g/lit, T_9-Control]

Table 3: Impact of seed priming on yield related traits of black cumin

Treatment	Number of seeds per capsule	Number of seeds per plant	1000 Seed Weight (g)	Straw yield (g)	Fresh Plant Weight (g)	Dry plant weight (g)	Seed Eight per plant (g)	Seed yield per plot (kg)	Projected seed yield (qha^{-1})	Projected oil yield (Lha^{-1})
T_1	78.46^{b}	1328.68	1.71^{a}	9.15^{bc}	18.45^{c}	10.22^{d}	3.60^{b}	0.71^{e}	7.19^{b}	2.81^{b}
T_2	80.00^{b}	1572.21	1.72^{a}	10.37^{abc}	31.71^{ab}	15.93^{abcd}	3.46^{b}	0.69^{e}	6.92^{b}	3.29^{b}
T_3	88.66^{ab}	1575.07	1.78^{a}	13.18^{ab}	32.76^{ab}	16.85^{abc}	3.39^{a}	0.67^{d}	6.78^{a}	2.93^{a}
T_4	86.90^{ab}	2045.75	1.95^{a}	15.11^{a}	37.68^{a}	21.44^{a}	4.16^{a}	0.83^{a}	8.32^{a}	4.19^{a}
T_5	87.83^{ab}	1909.82	1.62^{a}	11.03^{abc}	27.15^{abc}	13.04^{bcd}	3.26^{b}	0.65^{f}	6.52^{b}	3.29^{b}
T_6	80.90^{b}	1724.90	1.73^{a}	10.61^{abc}	26.51^{bc}	15.85^{abcd}	3.51^{b}	0.70^{e}	7.01^{b}	2.63^{b}
T_7	87.56^{ab}	1829.60	1.55^{ab}	11.50^{abc}	32.38^{ab}	18.42^{ab}	3.34^{a}	0.66^{c}	6.68^{a}	3.18^{a}
T_8	93.03^{a}	1796.17	1.93^{a}	14.30^{a}	36.53^{ab}	18.55^{ab}	3.73^{a}	0.74^{b}	7.46^{a}	4.59^{a}
T_9	80.76^{b}	1355.18	1.33^{b}	6.74^{c}	19.98^{c}	11.06^{cd}	3.02^{b}	0.60^{g}	6.04^{b}	2.02^{b}
S. Em (±)	3.23	168.12	0.07	1.42	3.21	1.86	0.08	0.01	0.17	0.43
C.D (P=0.05)	NS	NS	NS	4.29	9.73	5.62	0.25	0.05	0.51	1.27

[T_1-Seed treatment with Salicylic acid @200ppm, T_2- Seed treatment with Chitosan @500ppm,T_3-Seed treatment with Carbendazim @2g /kg, T_4- Seed treatment with Salicylic acid @200ppm + Carbendazim spray@ 2g/lit at 30DAP,T_5- Seed treatment with Chitosan @500ppm + Carbendazim spray@ 2g/lit at 30DAP, T_6-Seed treatment with Salicylic acid @200ppm + *Trichoderma viridae* @ 2g/lit spray at 30DAP, T_7-Seed treatment with Chitosan @500ppm + *Trichoderma viridae* @ 2g/lit spray at 30DAP, T_8-Seed treatment with *Trichoderma viridae*@ 2g/lit, T_9-Control]

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