



Effect of Biofertilizer, Microalgae and *Nigella sativa* on Purple Blotch (*Alternaria porri*) of Onion

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Onion (*Allium cepa* L.) is a biennial bulb crop produced all over the world. It is attacked by many diseases, out of which purple blotch of onion caused by *Alternaria porri* is the major disease. It causes heavy yield loss in both bulb production and seed production. The experiment was conducted to evaluate the effect of Biofertilizer, Microalgae and *Nigella sativa* on Purple blotch (*Alternaria porri*) of Onion. The results revealed that minimum disease incidence was observed at 45 and 60 days after transplanting in T₇ Carbendazim (8.72%, 5.72%), followed by T₆ *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (21.47%, 16.88%), T₄ Microalgae (23.54%, 17.21%) and

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maximum in T₃ *Nigella sativa* (31.45%, 21.75%). The maximum plant height (cm) at 45 and 60 days after transplanting was observed in T₆ *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (45.21cm, 57.73cm), followed by T₄ Microalgae (45.03cm, 57.46cm) and least in T₃ *Nigella sativa* (36.23cm, 51.36cm). The maximum length of leaves (cm) at 45 and 60 days after transplanting was observed in T₆ *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (45.21cm, 61.28cm), followed by T₄ Microalgae (45.03cm, 60.65cm) and least in T₃ *Nigella sativa* (36.23cm, 52.70cm). The maximum bulb weight (gm) and bulb size (cm) was observed in T₆ *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (67.25gm, 6.76cm), followed by T₄ Microalgae (64.30gm, 6.30cm) and least in T₃ *Nigella sativa* (54.39gm, 4.45cm).

Keywords: *Alternaria porri*; *Azotobacter*; microalgae; *Nigella sativa*; rhizobium.

1. INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important vegetable crops commercially grown in the world. It probably originated from Central Asia between Turkmenistan and Afghanistan, where some of its relatives still grow in the wild. Onion from Central Asia, the supposed onion ancestor had probably migrated to the Near East.

Among the vegetables, onion often called as "Queen of kitchen" (Selvaraj, 1976). It is one of the oldest known vegetable grown in India. It is commonly used for cooking purpose by all the communities of people. It ensures excellent taste to dishes and also exhibits a number of therapeutic properties such anti-bacterial, anti-fungal, anti-helminthic, anti-inflammatory, antiseptic, antispasmodic, etc.

Onion is attacked by many diseases and insect pests. The pathogen can infect the crop both in field and storage conditions. *Alternaria* blight disease is one of the most prominent diseases caused by the genus *Alternaria* with average yield loss of 32-57%. In onion crop 62% disease incidence was found in *Kharif* season (April-October month) while 38% disease incidence was recorded in *Rabi* season (October-March month). However, losses of about 80 to 85% have been observed on the crop (Mustafijur Rahman et al., 2015; Singh et al., 2021)

Appropriate management practice of purple blotch of onion has become an issue in present condition. Hence this study aimed to investigate the effect of biofertilizers, microalgae, and *Nigella sativa* on purple blotch (*Alternaria porri*) in onions. Moreover, this research focused on eco-friendly bio-agents, including *Azotobacter* sp., *Rhizobium* sp., and *Nigella sativa* extracts, evaluated through in-vitro screening for the management of purple blotch in onions.

2. MATERIALS AND METHODS

The present research work was conducted during *Rabi* 2019-20 in the Central Research Field of the Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh. The experiment was conducted in Randomized Block Design with seven treatments and three replications. Eight treatments were randomly arranged in each replication divided into twenty four plots and each plot size was 2 x 1 m.

2.1 Collection, Isolation and Identification of the Pathogen

The infected sample of *Alternaria porri* of onion was collected from research plot of onion at the central field of SHUATS, Prayagraj (U.P.) during February 2020. The standard tissue isolation technique was followed to isolate the pathogen. Small pieces of tissues about 3 mm from infected collar region with some healthy tissues were cut with sterile scalpel. Microscope was used for the examination of morphology and culture characteristics of fungal structures. The tissues were surface sterilized with 1% Sodium Hypochlorite solution for 30 sec. The tissues were subsequently washed thrice in sterile distilled water to eliminate excess Sodium Hypochlorite and then pieces were transferred to PDA plated petri dishes in presence of laminar air flow chamber. Plates were incubated at 28 ± 2°C and were observed periodically for growth of the fungus (Plate 1). The conidia of *Alternaria porri* was obclavate, borne singly on the tip of conidiophores, rarely in chains. The main body of the conidium was brown, transversely as well as longitudinally septate, number of transverse septa varied in the range of 10-12. The beak was sub-hyaline, simple or forked (Plate 1) (Rands, 1917).

Table 1. Detail of treatment

Sr. No.	Treatment No.	Treatment Details
1.	T ₀	Control
2.	T ₁	<i>Rhizobium</i>
3.	T ₂	<i>Azotobacter</i>
4.	T ₃	<i>Nigella sativa</i>
5.	T ₄	Microalgae
6.	T ₅	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i>
7.	T ₆	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i> + Microalgae
8.	T ₇	Carbendazim

2.2 Isolation of *Azotobacter*

Azotobacter was isolated using serial dilution Procedure- Take 10 gm of Rhizospheric soil of cereal crop. Added it to 90 ml of distilled water and mixed it well. Carried serial dilution from 10⁻¹ to 10⁻⁷ in sterilized conical flasks. Poured Jense's media in petri plates and allowed it to solidify. Incubated it in BOD at 28°C for 3 days (Plate 2) (Tabassum & Raj, 2016).

2.3 Isolation of *Rhizobium*

Detached the nodules from root system of legume crop and placed them in petriplates containing 75% Alcohol solution for 3 min. Rinsed them in petri plates containing water for 3-4 times. Collected 1-2 nodules in a test tube containing 1 ml of distilled water. Crushed the nodules and exudate was made. Took 1-2 loopful of exudate in a test tube containing 1 ml of distilled water. Transferred 1 ml of exudate in petri plate containing YEMA media. Incubated it in BOD at 28°C for 4-5 days (Plate 3) (Hamza & Alebejo, 2017).

2.4 Morphological Characterization of *Azotobacter* and *Rhizobium*

List 1. Characterization of *Azotobacter* and *Rhizobium*

Character	<i>Azotobacter</i>	<i>Rhizobium</i>
Cell shape	Mucoid	Rod shaped
Surface	Flate	Smooth
Colour	Milky colonies	Off white, creamish

2.5 Biochemical Characterization of *Azotobacter* and *Rhizobium* Isolates According to Bergey's Manual

After phenotypic identification of the isolates, they are further proceeded to various classical biochemical tests in order to obtain the accurate

identification of gram-negative rod bacteria. Biochemical tests (Gram Staining, Methyl Red Test, Indol Test, Catalase Test, Urease Test, Triple Sugar Test) for identification were done following the Bergey's manual of Determinative Bacteriology (1994). Old cultures were used and each test was conducted with three replicates for all biochemical tests. The biochemical tests were carried out with appropriate controls following the standard procedures.

2.6 Gram's Reaction

This test is essential to differentiate bacteria into gram-positive bacteria and gram-negative bacteria. A loopful of bacterial suspension was smeared on a glass slide. It was air-dried and heat fixed. The smear was flooded with crystal violet solution for 1 min. The slide was washed with a gentle stream of tap water blot dried and flooded with Lugol's iodine for 1 min. Again, the slide was washed with water and blot dried and then decolorized by washing in a gentle stream of 95% Ethyl Alcohol for 30 sec. to remove excess stain that easily washed away, counter staining was done by flooding with Safranin for 20 sec. The slide was again washed with tap water and blot dried. The preparation was observed under compound microscope at different magnifications. The gram-negative bacteria appeared pinkish-red in colour (Plate 4-A and 4-B). However, gram-positive bacteria appear blue-violet in colour (Roychowdhury *et al.*, 2017).

2.7 Catalase Test

24 hrs. old slant culture of *Azotobacter* and *Rhizobium* isolates was flooded individually with 1 ml of 3% Hydrogen Peroxide (H₂O₂) and observed the *Azotobacter* and *Rhizobium* isolates for the production of gas bubbles. The effervescence indicated the positive catalase activity (Plate 5-A and 5-B) (Bedi & Naglot, 2011).



Plate 1. Pure culture and microscopic view of *Alternaria porri*

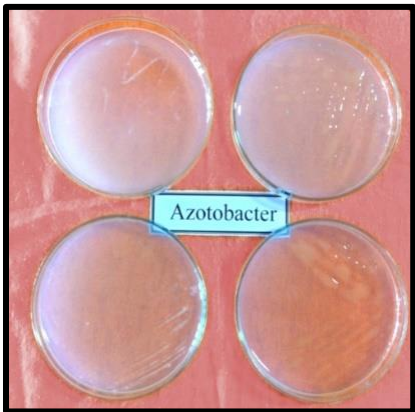


Plate 2. *Azotobacter* sp

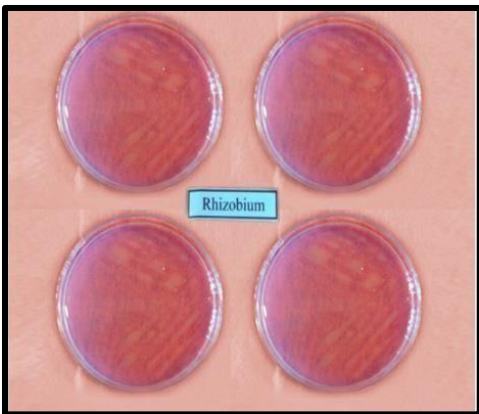


Plate 3. *Rhizobium* sp.

Table 2. Isolation and Identification of *Azotobacter* and *Rhizobium* isolates through Biochemical Tests

Biochemical Test	<i>Azotobacter</i> isolates	<i>Rhizobium</i> isolates
Gram's reaction	Negative	Negative
Catalase test	+	+
Indole test	+	+
Methyl red test	+	+
Urease test	+	+
Triple sugar test	+	+

+ Positive; - Negative

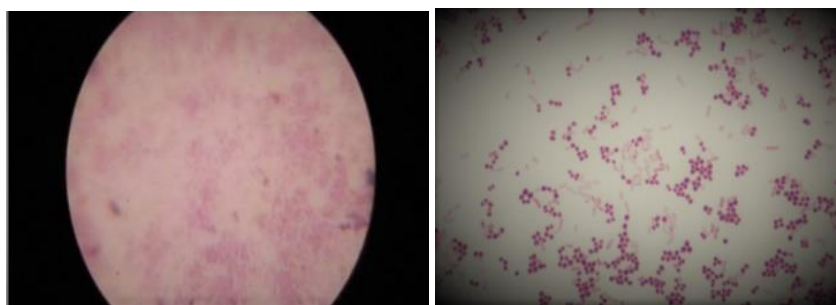


Plate 4. A Microscopic view of *Rhizobium* Plate. 4-B Microscopic view of *Azotobacter*



Plate 5. A Catalase Test of *Azotobacter* Plate 5. B Catalase Test of *Rhizobium*



Plate 6. Methyl Red Test of *Azotobacter* and *Rhizobium*

2.8 Methyl Red Test

The Methyl Red Voges Proskauer broth was prepared. 5 ml of the broth was poured into the sterile test tubes. The *Azotobacter* and *Rhizobium* isolates were inoculated separately into the test tubes and incubated at $28 \pm 2^\circ\text{C}$ for 2 days. After the incubation period, 5 ml of Methyl Red indicator was added to each test tube. Red colouration of the broth designated the positive result (Plate 6). However, the negative result is indicated by yellow colouration (Nagananda et al., 2010).

2.9 Urease Test

Christensen's urea agar medium was prepared. The medium was poured into the sterile test tubes and allowed to solidify. The *Azotobacter*

and *Rhizobium* isolates were inoculated separately into the test tubes and incubated at $28 \pm 2^\circ\text{C}$ for 4 days. After the incubation period, the appearance of deep pink color indicated the positive results (Plate 7) (Nagananda et al., 2010).

2.10 Triple Sugar Test

Triple sugar iron agar medium was poured into the sterile test tubes and allowed to solidify. The *Azotobacter* and *Rhizobium* cultures were inoculated into the test tubes and were incubated at $28 \pm 2^\circ\text{C}$ for 24 hrs. The results were noted (Plate 8) (Nagananda et al., 2010).

2.11 Preparation of *Nigella sativa* extract

The seed material was accurately weighed 5 gm. in an extraction thimble and a piece of



Plate 7. Urease Test of *Azotobacter* and *Rhizobium*



Plate 8. Triple Sugar Test of *Azotobacter* and *Rhizobium*

cotton was placed on the top of the thimble to distribute the solvent as it drops on the sample. The rapped sample was placed in the extractor. The empty receiving flask was weighed and about 250ml of n-hexane was added and attached to the extractor having thimble with seed material. The flask was heated on electrical heating mantle. The volume of the solvent was maintained by addition of solvent to compensate the loss due to evaporation. The extraction was continued for 3 hrs. It was allowed to cool down and extractor was disconnected. The thimble was taken out. This cycle was repeated to achieve complete extraction of oil (Khoddami et al., 2011).

2.12 Disease Incidence

Disease incidence was calculated by the following formula (Singh et al., 2021).

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

3. RESULTS AND DISCUSSION

3.1 Effect of Biofertilizer, Microalgae and *Nigella sativa* on Disease Incidence of *Alternaria porri* of Onion at Different Time Intervals

The data presented in Table 3 and depicted in Fig. 1 reveals the effect of biofertilizer, microalgae and *Nigella sativa* on disease incidence (%) of *Alternaria porri* of onion at 45 and 60 days after transplanting under field condition.

At 45 and 60 days after transplanting minimum disease incidence was observed in T₆ - *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (21.47% and 16.88% respectively), followed by T₄ - Microalgae (23.54% and 17.21% respectively), T₅ - *Rhizobium* + *Azotobacter* + *Nigella sativa* (27.42% and 19.95% respectively) and maximum disease incidence was in T₃ -

Nigella sativa (31.45% and 21.75% respectively) as compared to control T_0 - (32.56% and 21.84% respectively) and check T_7 - Carbendazim (8.72% and 5.72% respectively).

3.2 Effect of Single and Combined Application of Biofertilizer, Microalgae and *Nigella sativa* on Plant Height (cm) of Onion at Different Time Intervals

The data presented in Table 4 and depicted in Fig. 2 reveals the effect of single and combined application of biofertilizer, microalgae and *Nigella sativa* on plant height (cm) of onion at days before transplanting, 45 and 60 days after transplanting under field condition.

At days before transplanting, the observed plant height (cm) of onion under field condition was T_6 (15.11), T_5 (14.84), T_1 (14.77), T_4 (14.52), T_7 (14.08), T_3 (14.03), T_2 (13.64), T_0 (12.68).

At 45 and 60 days after transplanting maximum plant height was observed in T_6 - *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (45.21cm and 57.73cm respectively), followed by T_4 - Microalgae (45.03cm and 57.46cm respectively), T_5 - *Rhizobium* + *Azotobacter* + *Nigella sativa* (43.94cm and 57.19cm respectively) and least plant height was in T_3 - *Nigella sativa* (36.23cm and 51.36cm respectively) as compared to control T_0 - (34.10cm and 45.18cm respectively) and check T_7 - Carbendazim (42.03cm and 53.38cm respectively).

3.3 Effect of single and Combined Application of Biofertilizer, Microalgae and *Nigella sativa* on bulb weight (g) and bulb size (cm) of Onion at 120 days After Transplanting

The data presented in Table 5 and depicted in Fig. 3 reveals the effect of single and combined application of biofertilizer, microalgae and *Nigella sativa* on bulb weight (gm) and bulb size (cm) of onion at 120 days after transplanting under field condition.

3.3.1 Bulb weight

At 120 days after transplanting, the maximum bulb weight of onion was observed in T_6 - *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (67.25gm), followed by T_4 -

Microalgae (64.30gm), T_5 - *Rhizobium* + *Azotobacter* + *Nigella sativa* (62.27gm) and least plant height was in T_3 - *Nigella sativa* (54.39gm) as compared to control T_0 - (50.85gm) and check T_7 - Carbendazim (60.30gm).

3.3.2 Bulb size

At 120 days after transplanting, the maximum bulb size of onion was observed in T_6 - *Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae (6.76cm), followed by T_4 - Microalgae (6.30cm), T_5 - *Rhizobium* + *Azotobacter* + *Nigella sativa* (6.13cm) and least plant height was in T_3 - *Nigella sativa* (4.45cm) as compared to control T_0 - (3.61cm) and check T_7 - Carbendazim (5.76cm).

The results revealed that amendments with (*Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae) significantly enhanced plant growth parameters such as plant height, length of leaves, bulb weight, bulb size, etc. and while minimize the disease incidence. Biofertilizers releases the macro- nutrients slowly and stimulate root growth by producing some hormones and antimetabolites, ensuring sustainable crop improvement.

A biofertilizer is a substance which contains living micro-organisms which promotes plant growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus thereby stimulating plant growth through the synthesis of growth promoting substances. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build the soil organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability health of the soil. Biofertilizers can be expected to reduce the use of synthetic fertilizers and pesticides, but they are not yet able to replace them. Since they play several roles, a preferred scientific term for such beneficial bacteria is "Plant Growth Promoting Rhizobacteria" (PGPR) studied by (Mahdi et al., 2010).

Studies on the use of microalgae have shown positive effect on plant growth. This is likely due to the presence of several molecules such as plant growth hormones (cytokines, auxins, abscisic acid and gibberellic acid), polysaccharides, betaines and micronutrients. Among biotic

stresses, microalgae showed antifungal activity against different pathogen of plants. Microalgae are thus being used in agriculture as soil amendments for their beneficial effects on plant health and productivity. Similar findings have been reported

by (Righini & Roberti, 2019). The use of bioresources has been found to be effective against plant pathogens and also effective in increasing plant height, length of leaves, bulb weight and size as reported by (Kumar *et al.*, 2019).

Table 3. Effect of Biofertilizer, Microalgae and *Nigella sativa* on disease incidence of *Alternaria porri* of onion at different time intervals

Treatments		Mean of three Replications	
		45 DAT	60 DAT
T ₀	Control	32.56	21.84
T ₁	<i>Rhizobium</i>	30.51	20.12
T ₂	<i>Azotobacter</i>	31.15	21.04
T ₃	<i>Nigella sativa</i>	31.45	21.75
T ₄	Microalgae	23.54	17.21
T ₅	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i>	27.42	19.95
T ₆	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i> + Microalgae	21.47	16.88
T ₇	Carbendazim	8.72	5.72
S. Ed. (±)		1.7	1.3
CD at (0.05%)		3.69	2.85

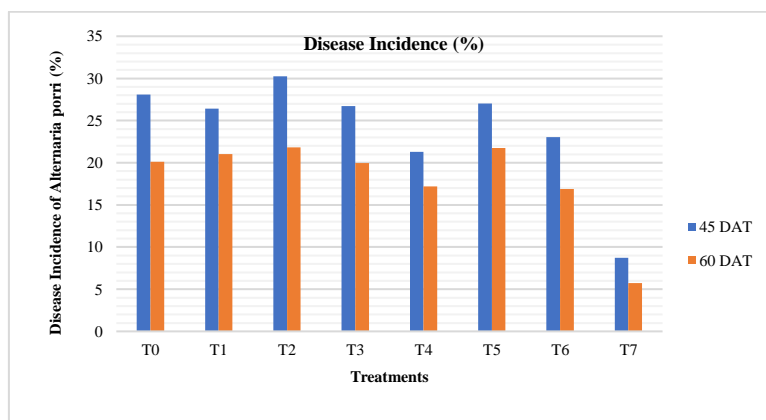


Fig. 1. Effect of Biofertilizer, Microalgae and *Nigella sativa* on disease incidence of *Alternaria porri* of onion at different time intervals

Table 4. Effect of single and combined application of Biofertilizer, Microalgae and *Nigella sativa* on plant height (cm) of onion at different time intervals

Treatments		Mean of three Replications		
		DBT	45 DAT	60 DAT
T ₀	Control	12.68	34.10	45.18
T ₁	<i>Rhizobium</i>	14.77	40.62	52.86
T ₂	<i>Azotobacter</i>	13.64	39.14	52.63
T ₃	<i>Nigella sativa</i>	14.03	36.23	51.36
T ₄	Microalgae	14.52	45.03	57.46
T ₅	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i>	14.84	43.94	57.19
T ₆	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i> + Microalgae	15.11	45.21	57.73
T ₇	Carbendazim	14.08	42.03	53.38
S. Ed. (±)		0.58	0.82	1.19
CD at (0.05%)		1.26	1.78	2.59

DBT- Days Before Transplanting
DAT- Days After Transplanting

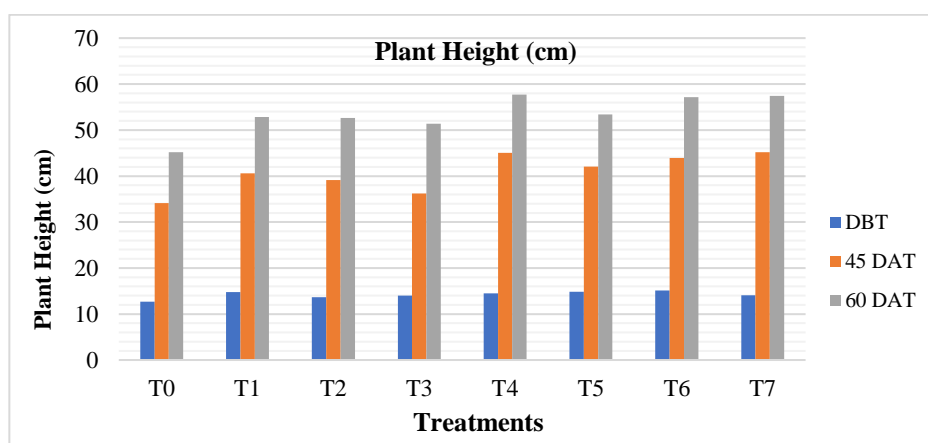


Fig. 2. Effect of single and combined application of Biofertilizer, Microalgae and *Nigella sativa* on plant height (cm) of onion at different time intervals

Table 5. Effect of single and combined application of Biofertilizer, Microalgae and *Nigella sativa* on bulb weight (gm) and bulb size (cm) of onion at 120 days after transplanting

Treatments		Mean of three Replications	
		Weight	Size
T ₀	Control	50.85	3.61
T ₁	<i>Rhizobium</i>	58.37	5.41
T ₂	<i>Azotobacter</i>	56.44	5.07
T ₃	<i>Nigella sativa</i>	54.39	4.45
T ₄	Microalgae	64.30	6.30
T ₅	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i>	62.27	6.13
T ₆	<i>Rhizobium</i> + <i>Azotobacter</i> + <i>Nigella sativa</i> + Microalgae	67.25	6.76
T ₇	Carbendazim	60.30	5.76
S. Ed. (±)		0.96	0.48
CD at (0.05%)		2.08	1.42

DAT- Days After Transplanting

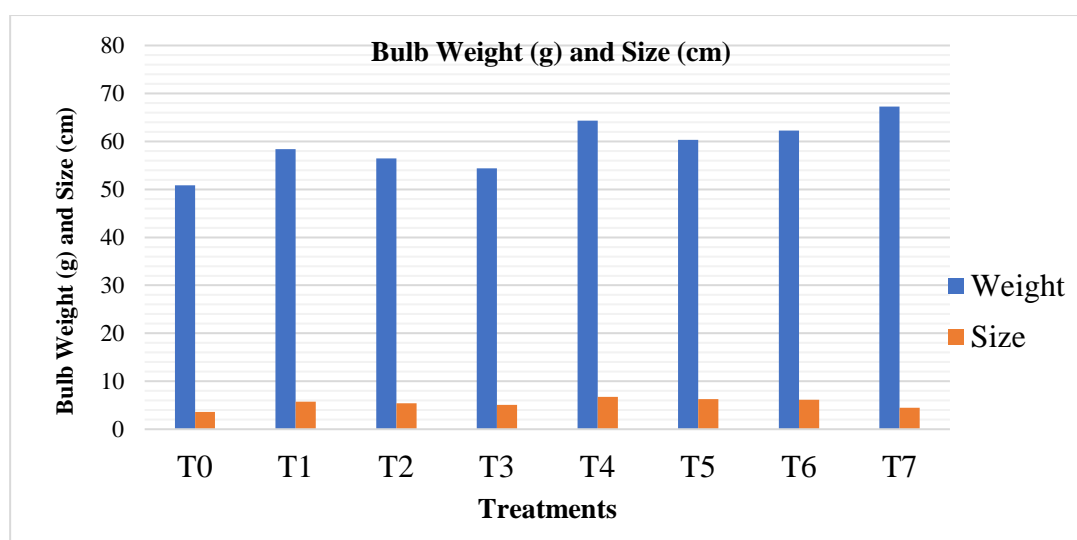


Fig. 3. Effect of single and combined application of Biofertilizer, Microalgae and *Nigella sativa* on bulb weight (g) and bulb size (cm) of onion at 120 days after transplanting

4. CONCLUSION

The present study reveals that the seedling treatment with T₆ (*Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae) is effectively working on purple blotch (*Alternaria porri*) of onion and has a great impact on various growth parameters. The onion seedlings treated with T₆ (*Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae) have obtained maximum plant height, maximum bulb weight and maximum bulb size. The same treatment has resulted in minimum disease incidence of *Alternaria porri*.

The use of inorganic chemicals is hazardous to consumers and it leaves the residues behind resulting in deterioration of soil health. Thus, it is concluded that the use of biological resources (*Rhizobium* + *Azotobacter* + *Nigella sativa* + Microalgae) is best for the management of purple blotch (*Alternaria porri*) of onion and for the growth enhancement of onion crop. However, the present study was limited to one crop season under Prayagraj agro-climatic conditions. Therefore, to substantiate the present results and to recommend the treatment more trials are needed.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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