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Study the effect of integrated disease management approach on germination, plant height and yield of potato and on severity of late blight of potato *Phytophthora infestans* (Mont.) de Bary.

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Abstract

Late blight of potato caused by Phytophthora infestans (Mont.) de Bary is one of the most serious diseases of potato. Several management practices have been adopted so for to minimize the disease. But in present investigation based on laboratory and glass house condition revealed that soil application and tuber seed dressing with bioformulation provided good protection against late blight and also stimulate the germination. The data showed that the plant height of potato was maximum in treatments T1(Soil application of FYM @ 125gm/pot +mushroom waste with T. harzianum @ 5gm/gm+tuber treatment with Azotobacter @ 5%+foliar spray with Ridomil @ 0.25%) with the value of 44.9 cm at 30 days age of plant. Tuber treatment with bio agents stimulated the germination per cent of the tuber. The Maximum germination per cent was noted in case of T1(Soil application of FYM @ 125gm/pot+mushroom waste with T. harzianum@ 5gm/kg+tuber treatment with Azotobacter@5%+foliar spray with Ridomil@0.25%). Growth promoting effects of the soil amendment+ tuber treatment + foliar spray were also perceived. The fungicides and bio agents were found to increase the plant length over control. Foliar spray with the fungicides pathogenic inoculation protected the plants against infection resulting reduced disease severity. The minimum disease severity (10.66) was reported from treatments were given as treatment T1(Soil application of FYM 125gm/pot+ mushroom waste with T. harzianum 5gm/kg +tuber treatment with Azotobacter @5%+foliar spray Ridomil @ 0.25%) treated plants against 82.33% disease severity in case of control. The integrated effect o FYM+ mushroom waste as soil application bioformulation as tuber 51.67gm, and total weight of tuber 338.33gm, where trea tment was given as soil application of FYM @125gm/pot+ mushroom waste with T. harzianum@ 5gm/kg+tuber treatment with T. harzianum @5%+foliar spray with 665.02gm was recorded from treatment T1(Soil application of FYM 125gm/pot +mushroom waste T. harzianum @ 5gm/kg+tuber treatment with Azotobacter@ 5%+foliar spray with Ridomil @ 0.25%).

Keywords: Late blight, Azotobacter, mushroom waste, Trichoderma

Introduction

Potato can be used as a vegetable alone or in combination with other vegetables such as cabbage, cauliflower, tomato, brinjal, beans etc. It is also used in the preparation of chips, puffs and raw pulp. It is served in meals as well as snacks preparation. Thus, potato is important for small- hold farmers as well as a cash crop. The daily need of potato is increasing day by day resulting from increasing population growth of the world. Therefore, there is a demand for more production from per capital available land. Though, production of potato is increasing continuously but productivity rate per unit area of land is very low as per other countries of the world, which has the main reason of biotic factors caused by fungi, bacteria, virus, viroid and nematodes etc. Among them late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary is one of the most serious and destructive diseases of potato all over world, including India. The great Irish famine in 1845, due to late blight is one of the most dramatic episodes caused by a plant pathogen in human history (Khurana *et al.*, 1998) [1]. The Irish famine affected food production as well as economic and social aspect of communities (Large, 1940) [2]. The disease is also the major bottleneck in potato production in Ethiopia and other parts of the world (Goodwin *et al.* 1995) [3].

The typical symptoms of late blight of potato appear at first as circular or irregular water-soaked spots, start usually at the tips or edges of the lower leaves. Tubers may appear shriveled as older lesions become firm and sunken due to water loss. The cotton like white mold may be observed on the surface of tubers when they are stored under conditions of high moisture. The pathogen perpetuates through soil and seed tubers through production of resting spore i.e. Oospore. Therefore, management of the disease can be done through host resistance, cultural adjustments, biological management. Cultural practice like field sanitation, summer ploughing, soil solarisation, soil amendments and crop rotation etc. can minimize the possibility of disease but cannot completely control the disease in standing crops. Other alternative method of disease management strategy is biological control. In this context, Trichoderma harzianum, Trichoderma viride, Chaetomium globosum, Gliocladium virens etc. have been exploited for management of diseases but biological control alone cannot manage the disease completely because a little fluctuation in temperature, pH, moisture etc. largely affects the efficacy of bio agent. Hence, the use of fungicides is the last and only method for management of plant disease. But continuous use of fungicides may develop resistant strain of the pathogen which has also adverse effect to human health. Continuous use of fungicides leads to increase in the development resistance population of *Phytophthora infestans* Mont.) De Bary.

The only one and single method is use of Integrated Disease Management which ultimately fulfils the customer desire to protect their livelihood. Various practices like cultural, chemical, biological control and use of resistant varieties have been used alone and in combination to manage the disease as reported by several workers (Singh, 1996; Joshi and Pundhir, 2013; Shaibala and Pundhir, 2008;) [4, 6, 5]. Mishra et al. (2016) [8] reported that integrated approach reduced the disease severity of early blight and increased crop yield of tomato. Muraj et al. (2016) also found that incorporation of biofertilizers in soil + tuber treatment with bio agents + foliar spray with bio formulation effectivity managed the late blight of potato. Therefore, keeping the above mentioned account in the mind, present investigation entitled "Study the effect of integrated disease management approach on germination, plant height and yield of potato and on severity of Late blight of potato Phytophthora infestans (Mont.) de Bary.

Materials and Methods

The present investigations based on Glasshouse experiment as well as laboratory were undertaken at Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology Kanpur during crop season 2016-18. The procedure and techniques applied during the course of investigations were elucidated as below:

Isolation, purification, identification and maintenance of *Phytophthora infestans* (Mont.) de Bary Collection of infected plant samples

Late blight infected leaves were collected from the potato field at Vegetable Research Farm, C.S. Azad University of Agriculture and Technology, Kanpur. Infected leaves with sporulation lesions were taken from the field and washed in sterilized water. The leaves were then placed in a humidity chamber with the leaf an axial side up. They were incubated at 18±1 °C in BOD, until sporulation appeared. Small pieces of infected tissue along with healthy portion from the sporulating border were cut and placed under potato slices in

empty sterilized Petri-plates. The Petri-plates were incubated at 18 ± 1 °C for 10 days until there was a growth of abundant mycelium on the upper side of the slice. Mycelium was taken from the tuber slices by using sterilized needle and transferred on the selective medium.

Preparation of culture media

The *Phytophthora infestans* (Mont.) de Bary causes late blight of potato belongs to Class Oomycetes and generally not grown on Potato Dextrose Agar Media. Therefore, following selective culture media are being used to isolate the fungus.

Tomato based specific media Tomato juice medium:

Tomato juice - 250ml
Calcium carbonate - 0.04g
Agar powder - 20gm
Distilled water - 1000ml
Dextrose - 20 g.

The tomato dextrose agar medium prepared was sterilized at 121.6 °C, 15 psi. For 15 minutes in an autoclave.

Procedure:

Fresh and healthy tomato were collected from market and washed thoroughly in running tap water and then distilled water to remove dust and foreign matter from the surface. 250gm tomato was taken and cut into the small pieces and grinding with electric mixer or Oster food blender. The obtained slurry was passed through a sieve with a pore size of 1.5×1.5mm to remove large pieces of tissues. The filtrate was measured in a measuring cylinder and final volume made up to 1lt by adding more distilled water. It was again poured in sauce pan and heated. 20gm agar powder was added slowly in heating juice. The solution was boiled for some time till it tends to solidify on cooling. The prepared media was then poured in four conical flask of about 200 ml. 10 ml of media was poured in 10 culture tubes. Both conical flask and culture tubes were plugged with non-absorbent cotton and mouth was wrapped with butter paper and rubber band. The culture tubes were placed vertically in wire baskets. The media in flask and culture tubes were then autoclaved at 15lb/inch²pressure (15 psi) for 20 minutes at 121.6 °C.

(2). Rye based media

Compositions of culture medium:

Rye grains 60.00g
Sucrose 15.00g
Beta-sitosterol 0.050g
Agar 15.00g
Distilled water 1000.00ml

Procedure

Sixty gram rye grains were soaked in distilled water at room temperature for 36hrs and supernatant was retained after decantation. The grains were then steamed for one hour. The steamed grains were filtered through double layer of muslin cloth and the filter was collected in flask. Rye seed were discarded. The filtered was added to original supernatant (liquid decanted from the grain at beginning). 15g sucrose, 15g agar and 0.050g Bita sitosterol were added and volume was adjusted to one litre by adding distilled water. The medium was autoclaved at 15 psi cm² for 20 minutes and stored at 4 °C for subsequent use. A pinch of Streptomycin

sulphate was also added. The antibiotic was added in cooled medium, which was shaken well before pouring in the plates.

3.2.3 Isolations of pathogen

A small piece of infected leaf from border of sporulating lesion along with some healthy green tissue was cut and dipped in mercuric chloride solution (0.1%) for 30 seconds rinsed in sterilized distilled water thrice and dried off with sterilized filter paper. The small pieces were then placed on tomato extract based media which was previously pour in sterilized in Petri plates. The plates were then incubated at 18±1 °C. The Petri plates were observed daily to find out the presence of mycelium around the leave bits. As soon as the mycelia growth is notices around the bits, the pathogen was purified by hyphal tip culture method.

3.2.4. Purification of Phytophthora infestans (Mont) de

The white mycelial bits of Phytophthora infestans (Mont) de Bary was removed from the margin of fungal colony and then transferred to another Petri-plate which was previously poured with sterilized tomato extract based medium. After purification, the pure culture of Phytophthora infestans (Mont) de Bary was transferred on slant medium and incubated at 15-18 °C in darkness till full growth. The culture was then transferred into the incubator at 10-12 °C for further

3.2.5 Identifications of Phytophthora infestans (Mont) de

The isolated pathogen was identified on the basis of its morphological and cultural characters and pathogenic behaviour towards the host. Phytophthora infestans (Mont) de Bary belong to the class Oomycetes. The vegetation is mycelium characterized by the absence of cross walls, along with both asexual and sexual reproduction occurs. The sporangiophores and sporangia emerge at asexual reproduction phase. The sporangia are lemon shape, measurement of 21-38µm×12-23µm. Sporangia develops at the end of these sporangiophores. Oospores are found at sexual reproduction. When mycelia of different mating types of the fungus grow together, one of them may from antheridia and the other oogonia. The oogonium grows through the antheridium, allowing fertilization. The fertilized oogonium develops into a thick-walled oospore, while the oospore is orange red, nearly round-shaped, measurement of 28-32µm. The pathogen was found to produce the characteristics leaf spot symptoms on the affected plants. The isolation pathogen was identified on the basis of its morphological and cultural characters and pathogenic behaviour towards the host.

3.2.6 Maintenance of the culture

After confirmation of isolated pathogen Phytophthora infestans (Mont.) de Bary The pure culture was transferred on media slant and maintain in the BOD at 10-12 °C for further study.

3.3. Pathogenicity test

The pathogenicity test of isolated fungus was conducted on healthy potato plants in order establish the pathogenic nature of the fungus. The pathogenicity was tested according to Koch's postulates (1882).

The earthen pots of 30cm diameter were taken to conduct the present experiment. Initially the pots were filled with sterilized soil and water was added to bring the soil under good tilt condition. The healthy tubers of potato variety Kufri Pukhraj were placed in these pots and were allowed to grow for one month. The homogenized spore suspension was prepared in sterilized water from 7 days old culture of Phytophthora infestans (Mont.) de Bary. The suspension was sprayed on one month old potato plants @ 2ml/plant. The inoculated plants were placed on the bench of glass house. After 2-3 days, the plants began to show the symptoms of blight. The inoculated plants showed pale to dark green spots occur at the leaf tips and margins that change into brown or black lesions lair. These lesions are not delimited in size and enlarged rapidly in a favourable weather. On the lower side of leaves, a white mildew appears on the surface of lesions where the pale land purplish tissues join. These symptoms confirmed that the blighting was caused by Phytophthora infestans (Mont.) de Bary.

Plan of work

Experimental Design	Completely Randomized Design
Season	Rabi
Crop	Potato
Replication (Pot experiment)	3
Tubers/Pot	30
Variety	Kufri Pukhraj
Treatments	10

Experiment

Collection of experiment plant materials

The commonly available plants were collected from vegetable Research Farm Chandra Shekhar Azad University of Agriculture and Technology, Kanpur.

Collection of bio-fertilizers

All these bio-fertilizers were collected from Department of Soil Science and Agriculture Chemistry (Microbiology) and Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur to conduct the present study. The experiment was conducted in the Glass house complex of Department of Plant Pathology, C.S. Azad University of Agriculture and Technology, Kanpur during Rabi season 2016-18.

Collection of seed tuber

Truly labelled potato seed tubers of variety 'Kufri Pukhraj" were obtained from Vegetable Research Farm C.S.A. University of Agriculture & Technology, Kanpur to conduct the experiment.

Climate

Geographical condition of Kanpur district comes under the sub-tropical and semi-arid zone at north India situated between latitudes ranging from 25°26 north and longitudes 79°31 to 80°34 East with on latitude of about 125.9m above mean sea level.

Solution preparation of fungicide

The fungicide spray were prepared by the following formula

Tuber seed treatment

Two seed tubers were placed in each jar containing require concentration of each solution separately for two hours. It was then used for sowing in pots.

Seed treatment with bio agent

The packets of *Azotobacter* containing 200gm inoculation were obtained from Department of Soil Science, C.S.A. University of Agriculture & Technology, Kanpur. Seed tubers were treated with *Azotobacter* @ 2g/10g of seed 10gm Jaggery was also added to make slurry and mixed it with seed tuber. Then the tubers were kept in shade for dry.

On the other hands, seed tubers were also treated with formulation of neem cake, mustard cake @ 25%, bioformulation of *Trichoderma viride*, *Trichoderma harzianum* of the tuber seed. The seed tubers were treated by dipping the tuber in prepared solution separately. The treatments were given 4 hours before the sowing of tuber.

Foliar spray with fungicides

At 45 days, plants inoculated with spore suspension of *Phytophthora infestans* (Mont) de Bary and after 45 hrs. Plants were sprayed with fungicide like Ridomil @0.25%, Mancozeb @ 0.25% and Carbendazim @ 0.05%, of the tuber seed.

Effect of integrated approaches on seed tuber germination percentage, plant height, disease severity and tuber yield

The experiment was conducted at the Glasshouse complex, Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur. The seed tubers of potato variety 'Kufri Pukhraj' were treated with bio formulations of Trichoderma harzianum, Trichoderma viride and Azotobacter separately and sown in 30cm earthen pots, which were previously filled with a mixture of sterilized sandy loam and farm yard manure in the ratio of 2:1. In each pot, one seed tubers were sown and watered regularly. Three replications per treatment and three pots were sown with untreated seed tubers served as control. The treatment were given as follows

- **T1.** = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25%
- **T2.** = Soil application of FYM @ 125 gm/pot + mushroom waste with *T. harzianum* @5 gm/kg + tuber treatment with *T. harzianum* @5 % + foliar spray with Mancozeb@0.25%.
- **T3.** = Soil application of FYM @ 125gm/pot + neem cake@125gm/pot+ tuber treatment with *T. viride* @5% + foliar spray with Mancozeb @0.25%.
- **T4.** = Soil application of FYM @ 125gm/pot + mustard cake@125gm/pot+ tuber treatment with *Azotobacter*@ 5% + foliar spray with Carbendazim @0.05%.
- **T5.** = Soil application of FYM @ 250 gm/pot + tuber treatment with *T. viride* @5% + foliar spray with Ridomil @0.25%.
- **T6.** = Soil application of FYM @ 250gm/pot + tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb @0.25%.

- **T7.** = Soil application of FYM @ 250gm/pot + tuber treatment with *Azotobacter*@ 5% + foliar spray with Ridomil @0.25%.
- **T8.** = Soil application of FYM @ 250gm/pot + foliar spray with Carbendazim @0.05%.
- **T9.** = Soil application of FYM @ 250gm/pot + mustard cake @125gm/pot+ foliar spray with Ridomil @0.25%.
- **T10.** = Soil application of FYM @ 250gm/pot (control).

Observations pertaining to the effect of different treatments were taken as follows.

- 1. Germination percentage (up to 10days after sowing)
- 2. Plant height (up to 30days)
- 3. Disease severity (45 days 52 days)
- 4. Yield (after harvesting)

Germination percentage

Seed tuber treated with different bio formulations was responsible for early breaking of seed tuber. The observation on germination of tuber was taken at every 24 hours up to 10 days. Germination percentage was calculated by use of following formula:-

Germination
$$\% = \frac{\text{Number of germinated seed tubers}}{\text{Number of total seeds}} \times 100$$

Pl nt height

For this purpose, three plants were selected randomly from tagged plots. The shoot height was measured (in cm) from the soil surface at basal portion of flag leaf with the help of meter scale. Three replication were kept for each treatment. The average of three plants height was divided by 3 for obtaining their mean to consider plant height.

Disease severity:

Inoculation with Phytophthora infestans (Mont) de Bary

At 45 days age, plants were inoculated with spore suspension of pathogen. The concentration of sporangia was maintained at 10^6 sporangia/ml. The spore suspension was prepared from seven days old culture of the pathogen. The homogenized, spore suspension were inoculated on the foliage of each plant. The plants were then covered with polythene bags for 48 hrs. To provide suitable moisture and humidity for growth and development of the pathogen.

Measurement of disease severity

Observations for measuring the disease severity were taken after 5 days of pathogen inoculation. The disease severity was recorded on a 0-9 scale. Ten leaves randomly selected from the pot for measurement of disease severity. The leaves with 1-9% infection received 1, 10% infection received 2, 11-25% infection received 3, 26-40% infection received 4, 41-60% infection received 5, 61-70% infection received 6, 71-80% infection received 7, 81-90% infection received 8, 91-100% infection received 9.

The disease severity of individual plants was calculated by following formula

Disease severity PDI =
$$\frac{\text{Sum of numerical rating}}{\text{Total number of leaves examined} \times \text{maximum rating}} \times 100$$

Tuber yield

To explore the possible effect of the Integrated Disease Management on tuber yield was observed and data were taken on the weight of total no tubers per treatment and number of large, medium and small tubers. We are categorise larger tuber>50gm, medium size of tuber 25-49.5gm and small size of tuber <25gm. Then yield of crop was calculated by taking weight of all tubers.

Experimental Finding

The investigation on carried out on "Studies on late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary". The details of the experiment on symptomatology, isolation, purification, pathogenicity test, identification, and Integrated Disease Management approaches were taken up as per technique described under material and methods. The experiments were carried out both *in-vivo* and *in-vitro* in Completely Randomized Design (CRD) with three replications for each treatment. The results of the experiment are presented below:

4. Collection, Isolation, Purification and Identification of Pathogen

4.1 Symptomatology

Symptoms appear at first as water-soaked spots, usually at the edges of the lower leaves. In moist weather, the spots enlarge rapidly and form brown, blighted areas with indefinite borders. A zone of white, downy mildew growth of 3 to 5 millimeters wide appears at the border of the lesions on the undersides of the leaves. Soon entire leaves are infected, die, and become limp. Under continuously wet conditions, all tender, above ground parts of the plants blight and rot away giving off a characteristic odor. Entire potato plants and plants in entire fields may become blighted and die in a few days or a few weeks.

In dry weather, the activities of the pathogen are slowed or stopped. Existing lesions stop enlarging, turn black, curl, and wither and no mycelial growth appears on the underside of the leaves.

When the weather becomes moist again the mycelial growth resumes its activities and the disease once again develops rapidly. Affected tubers at first show purplish or brownish blotches consisting of water-soaked, dark, somewhat reddish brown tissue that extends 5 to 15 millimeters into the flesh of the tuber. Later the affected areas become firm and dry and somewhat sunken. Such lesions may be small or may involve almost the entire surface of the tuber without spreading deeper into the tuber interior. The rot, however, continues to develop after the tubers are harvested. Infected tubers may be subsequently covered with sporangiophores and spores of the pathogen or become invaded by secondary fungi and bacteria, causing soft rots and giving the rotting potatoes a putrid, offensive odor.

4.2 Isolation and identification of the pathogen

The isolation pathogen was identified on the basis of its morphological and cultural characteristic as described by Akhtar *et al.*, $(2005)^{[9]}$. The sporangia are multinucleate (7-30 nuclei), thin-walled, hyline, oval or pear shaped with a definite papilla at the apex. They measure 22-33µm×16-24µm. Sporangia develop at the end of these sporangiophores. The fertilized oogonium develops into a thick-walled oospore, while the oospores are orange red, nearly round-shaped, measurement of 28-32µm (Fig. 1).

4.3 Pathogenicity test

The pathogenicity test of isolated fungus was conducted on healthy potato plants in order to establish the pathogenic nature of the fungus. The pathogenicity was tested according to Koch's postulates (1882). The earthen pots of 30cm diameter were taken to conduct the present experiment. Initially the pots were filled with sterilized soil and water was added to bring the soil under good tilth condition. The healthy tubers of potato variety Kufri Pukhraj were placed in these pots and were allowed to grow for one month. The homogenized spore suspension was prepared in sterilized water from 7 days old culture. The suspension was sprayed on one month old potato plants @ 2 ml/plant. The inoculated plants were placed on the bench of glass house. After 2-3 days, the plants began to show the symptoms of blight. The inoculated plants showed pale to dark green spots occur at the leaf tips and margins the change into brown or black lesions later. These lesions are not delimited in size and enlarged rapidly in a favorable weather. On the lower side of leaves, a white mildew appears on the surface of lesions where the pale and purplish tissues join. These symptoms confirmed that the blighting was caused by Phytophthora infestans (Mont. de Barry). (Fig: 2). Re-inoculations were made from infected plant and culture was compared with original cultures to confirm the identity and pathogenicity of the pathogen.

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Effect of Integrated Disease Management Approach on Germination and Growth Parameter of Potato Plants 4.5.1 Tuber Germination

Seed tuber was treated with different fungicides and bio agents might be responsible for early breaking of seed tuber dormancy, thereby enhancing germination per cent of seed tuber. The observation on germination per cent of tuber was taken at every 24 hours up to 10 days. The data presented in the table 7 shows that 100 per cent germination was recorded T1 treatment, in which treatment was given as soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25% which was followed by T2

treatment (Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb @0.25%. with germination per cent 72.72% and T3 treatment (Soil application of FYM @ 125gm/pot + neem cake @125gm/pot+ tuber treatment with *T. viride* @5% + foliar spray with Mancozeb @0.25%) with germination per cent 88.88%. From the (Table-7), it is cleared that among the treatment least number of bud and germination per cent was found in case of T8, T9 and T10 respectively, representing 85.71per cent, 50per cent and 53.84per cent.

4.5.2 Plant height

The effect of seed treatment with bio-formulation on plant height of potato was studies under Glass house complex in pot culture experiment. The observation on plant height was taken at each day up to 30 days after sowing. The data represented in table 8 showed that the plant height was maximum in treatment T1 (Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @ 5gm/kg + tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @ 0.25%) with the value of 44.9 cm at 30 day age of plant followed by treatment T2 (Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @ 5gm/kg + tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb @0.25%), T3 (Soil application of FYM @ 125gm/pot + neem cake @125gm/pot + tuber treatment with *T. viride* @5% + foliar spray with Mancozeb @0.25%) representing value of 43.6cm and 42.5cm, respectively. From the table, it is also cleared hat all the treatments were able to increase the growth of plant over control (Fig: 5).

Table 1: Effect of Integrated Disease Management practices on germination of seed tuber

Treatment	Before Sowing Germination Total No Tuber Bud	After Sowing Germination Total No of Tuber Bud	Germination %
T1	12	12	100
T2	11	8	72.72
Т3	9	8	88.88
T4	13	8	61.53
T5	15	9	60.00
T6	9	7	77.77
T7	11	4	36.36
T8	7	6	85.71
Т9	10	5	50.00
T10	13	7	53.84
CD (0.05)	5.06	3.61	9.13
SE(d)	2.41	1.72	4.35
C.V.%	26.8	28.5	7.86

Whereas

- **T1.** = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25%.
- **T2.** = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb@0.25%.
- **T3.** = Soil application of FYM @ 125gm/pot + neem cake@125gm/pot+ tuber treatment with *T. viride* @5% + foliar spray with Mancozeb @0.25%.
- **T4.** = Soil application of FYM @ 125gm/pot + mustard cake@125gm/pot+ tuber treatment with *Azotobacter*@ 5% + foliar spray with Carbendazim @0.05%

Table 2: Effect of concentration treatments on plant height of potato at different days (1-15 days after sowing)

	Plant Height (in cm)														
Treatments	Day1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
T1	1.6	1.8	2.2	2.5	3.2	3.8	4.4	5.1	5.7	6.6	7.5	9.2	10.4	11.8	13.1
T2	1.5	1.5	1.8	2.4	2.8	3.8	4.3	4.8	5.5	6.3	7.4	8.6	10.3	11.4	12.7
T3	1.2	1.3	1.5	2.0	2.4	3.2	3.7	4.4	5.1	5.8	7.2	8.3	9.4	10.5	12.1
T4	1.4	1.0	2.2	2.2	2.3	3.1	3.6	4.1	4.8	5.9	6.8	7.5	9.1	10.4	11.9
T5	1.4	1.7	2.1	2.1	2.3	3.2	3.4	4.0	4.8	5.1	6.2	7.4	8.9	10.0	11.4
T6	1.2	1.4	1.5	1.9	2.4	2.8	3.8	4.4	4.7	5.0	6.3	7.2	8.6	9.8	11.0
T7	2.6	2.8	3.3	3.0	7.2	4.8	5.8	6.2	7.3	8.2	9.1	10.8	11.9	12.8	12.9
T8	1.8	2.5	2.7	3.1	3.8	4.5	4.5	5.2	6.5	7.0	8.2	9.1	10.8	11.8	12.7
T9	1.0	1.3	1.5	1.8	2.1	2.4	2.7	3.1	3.3	4.4	5.2	6.2	7.3	8.5	9.8
T10	1.4	1.6	2.1	2.5	3.2	3.9	4.6	5.8	6.2	7.1	7.8	8.5	9.9	10.3	11.0
C.D.(0.05)					0.83					0.25					0.87
S.E.(D)					0.43					0.12					0.41
C.V.%					16.8					2.41					4.3

Fig 3: Effect of different treatments on plant height of potato at different days (16-30after sowing)

	Plant Height (in cm)														
Treatments	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
T1	15.1	17.9	18.9	20.2	22.7	25.5	27.5	29.6	31.9	33.9	35.8	38.1	40.2	42.3	44.9
T2	14.3	16.2	17.8	19.2	21.4	23.9	26.5	28.2	30.5	32.7	34.8	37.1	39.2	41.6	43.6
T3	13.7	15.9	17.6	19.4	21.2	23.5	25.8	27.8	30.4	32.5	34.5	36.9	38.2	41.2	42.5
T4	13.5	14.8	16.5	18.4	20.2	22.5	24.6	26.6	28.1	30.0	32.09	34.9	36.8	38.5	39.9
T5	12.9	14.4	15.8	17.4	19.0	21.5	23.5	25.1	27.5	30.0	32.4	34.5	35.9	37.5	38.9
T6	12.6	14.0	15.7	17.6	18.9	20.9	23.1	25.5	27.1	29.8	32.1	33.8	35.0	37.1	38.4
T7	14.4	16.5	18.8	20.1	22.5	24.0	25.9	27.9	29.1	30.9	32.1	33.2	34.4	36.2	37.1
T8	13.5	15.2	17.6	19.8	21.5	23.5	25.5	27.3	28.9	29.9	31.9	32.1	33.0	34.6	35.9
T9	11.1	12.5	14.2	15.8	17.5	19.7	21.9	23.8	25.9	27.1	29.4	30.8	31.5	33.5	34.8
T10	12.4	14.4	16.8	18.4	20.5	22.5	24.7	26.5	27.7	29.0	29.9	31.8	32.0	32.8	33.5
C.D.(0.05)					2.50					7.79					1.98
S.E.(D)					1.19					3.71					0.94
C.V.%					7.17					2.97					2.97





Fig 1: Effect of tuber seed treatments with fungicides and bio agents on growth parameters of potato

- **T1.** = Soil application of FYM @ 250gm/pot + tuber treatment with *T. viride* @5% + foliar spray with Ridomil @0.25%.
- **T2.** = Soil application of FYM @ 250gm/pot + tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb @0.25%.
- **T3.** = Soil application of FYM @ 250gm/pot + tuber treatment with *Azotobacter*@ 5% + foliar spray with Ridomil @0.25%.
- **T4.** = Soil application of FYM @ 250 gm/pot + foliar spray with Carbendazim @0.05%.
- **T5.** = Soil application of FYM @ 250gm/pot + mustard cake @125gm/pot+ foliar spray with Ridomil @0.25%.
- **T6.** = Soil application of FYM @ 250gm/pot (control).

4.6 Effect of Integrated Disease Management practices on development of disease

The effect of tuber treatment with fungicide and bio agent on severity of disease revealed that there is a decline in late blight severity due to various treatments (Table-9). The susceptible variety Kufri Pukhraj of potato showed a 82.33%, 70.33% and 56.33 severity in case of Phytophthora infestans(Mont. de Barry) treated plants at 21, 14 and 7 days of inoculation. The minimum late blight severity was recorded in treatment T1 (soil application of FYM @ 250gm/pot + tuber treatment with Azotobacter @ 5% + foliar spray with Ridomil @0.25%) with value of 10.66%, 13.67% and 17.33% at 7, 14 and 21 days after inoculation, which was followed by treatment T2 (soil application of FYM @ 250gm/pot + tuber treatment with T. harzianum @ 5% + foliar spray with T. harzianum @ 5%), representing the value 11.66%, 14.66 and 18.33%. The rest of the treatments are also showing superior in reducing disease severity over control but inferior over treatment T1 and T2. The decrease in disease severity might be the activity of integrated effect of fungicide and bio agent which stimulate to synthesis of some defense compound in potato against Phytophthora infestans (Mont. de Barry). Similar results have also seen found in case of 14 and 21 days of observation. From the table it is also cleared that the severity continuously increases from 7 to 21 days in all treatments.

Table 4: Effect of Integrated Disease Management practices on severity of late blight of potato

Treatment	Disease severity %							
Treatment	7 Days	14 Days	21 Days					
T1	10.66	13.67	17.33					
T2	11.66	14.66	18.35					
Т3	12.13	15.33	19.67					
T4	13.33	16.00	20.34					
T5	14.66	16.33	21.33					
T6	15.00	17.67	22.33					
T7	15.66	18.67	23.33					
T8	16.33	19.33	24.35					
Т9	16.66	20.33	25.30					
T10	56.33	70.33	82.33					
CD (0.05)	10.82	9.94	11.77					
SE(d)	2.15	4.73	5.60					
C.V.%	34.6	26.1	24.9					

Whereas

- **T1.** = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25%.
- **T2.** = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb@0.25%.
- T3. = Soil application of FYM @ 125gm/pot + neem cake @ 125gm/pot + tuber treatment with T. viride @5% + foliar pray with Mancozeb @0.25%.
- **T4.** = Soil application of FYM @ 125gm /pot + mustard cake @ 125gm /pot+ tuber treatment with *Azotobacter*@ 5% + foliar spray with Carbendazim @0.05%.
- T5. = Soil application of FYM @ 250gm/pot + tuber treatment with T. viride @5% + foliar spray with Ridomil @0.25%.
- **T6.** = Soil application of FYM @ 250gm/pot + tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb @0.25%.
- T7. = Soil application of FYM @ 250gm/pot + tuber treatment with Azotobacter@ 5% + foliar spray with Ridomil @0.25%.
- **T8.** = Soil application of FYM @ 250gm/pot + foliar spray with Carbendazim @0.05%.
- $\textbf{T9.} = Soil \ application \ of \ FYM \ @ \ 250gm/pot + \ mustard \ cake \ @ \ 125gm/pot + \ foliar \ spray \ with \ Ridomil \ @ \ 0.25\%.$
- **T10.** = Soil application of FYM @ 250gm/pot (control).

4.7 Effect of integrated disease management practices on tuber size and yield of potato

The effect of Integrated Disease Management practice on tuber size and yield was studied after harvesting. Tubers were graded as large (more than 50gm), medium (25-49.5gm) and small (less than 25gm) (Table-10). It has found that maximum number of large tuber with 1 was found in T2 treatment, representing total tuber 53.46gm, where treatment was given

as soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg + tuber treatment with *T. harzianum* @5% + foliar spray with Mancozeb @0.25%. It is also cleared from the table that there is no large size tuber in rest of the treatment. The maximum number of medium size tuber with 10 was found in T2 treatment, representing with weight of tuber 343.24gm.On the other hand, the maximum number of small size tuber was found in T3 treatment representing with weight of tuber 527.7gm, where treatment was given as soil application of FYM @ 250gm/pot + tuber treatment with mustard cake + foliar spray with Mancozeb @0.25%.

As per yield concerned, the highest yield (668.6gm) was recorded from treatment T1 (soil application of FYM @ 250gm/pot + tuber treatment with *Azotobacter* @ 5% + foliar

spray with Ridomil @0.25%) followed by treatment T2 (Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *T. harzianum* @5% + foliar spray with Mancozeb @0.25%) and T3 (Soil application of FYM @ 125gm/pot + neem cake@125gm/pot+tuber treatment with *T. viride* @5% + foliar spray with Mancozeb @ 0.25%), representing the value of 640.02gm, 607.38gm, respectively. Among the treatment minimum yield was recorded in T9 treatment which is 344.14gm/pot against 343.04gm/pot in case of control. From the table in the cleared that all the treatments were able to increase the yield over control and the per cent increase of yield was varied from 3.43 to 90.46 per cent.

Table 5: Effect of Integrated Disease Management practices on tuber size and yield of potato

Tuestanism	Large 50 > gm		Medium 25-49	gm	Small 25 < g	m	Total yield	% Increase yield	
Treatment	Total No. of tuber	Weight	Total No. of tuber	Weight	Total No. of tuber	Weight	(gm/plant/pot)	over control	
T1	0	0.00	08	200.67	40	465.67	665.02	90.46	
T2	1	51.57	11	338.3	21	245.34	635.33	81.87	
Т3	0	0.00	03	80.33	56	529.67	611.99	74.62	
T4	0	0.00	04	111.67	34	327.68	439.32	25.76	
T5	0	0.00	02	63.33	40	338.33	401.67	14.98	
T6	0	0.00	02	150.67	28	238.33	388.99	11.35	
T7	0	0.00	05	53.35	31	311.67	364.99	4.48	
Т8	0	0.00	02	150.67	34	210.67	361.32	3.43	
T9	0	0.00	02	96.33	24	256.67	352.99	1.05	
T10	0	0.00	05	140.68	22	208.66	349.32		
CD (0.05)	0.54	1.57	4.96	57.09	7.83	9.26	28.00		
SE(d)	026	0.75	2.36	27.17	3.73	4.41	13.33		
C.V.%	31.6	17.7	55.9	24.00	13.9	1.72	3.61		

- T1. = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25%.
- **T2.** = Soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg+ tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb@0.25%.
- **T3.** = Soil application of FYM @ 125gm/pot + neem cake@125gm/pot+ tuber treatment with *T. viride* @5% + foliar spray with Mancozeb @0.25%.
- **T4.** = Soil application of FYM @ 125gm/pot + mustard cake@125gm/pot+ tuber treatment with *Azotobacter*@ 5% + foliar spray with Carbendazim @0.05%.
- T5. = Soil application of FYM @ 250gm/pot + tuber treatment with T. viride @5% + foliar spray with Ridomil @0.25%.
- **T6.** = Soil application of FYM @ 250gm/pot + tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb @0.25%.
- T7. = Soil application of FYM @ 250gm/pot + tuber treatment with Azotobacter@ 5% + foliar spray with Ridomil @0.25%.
- **T8.** = Soil application of FYM @ 250gm/pot + foliar spray with Carbendazim @0.05%.
- **T9.** = Soil application of FYM @ 250gm/pot + mustard cake @125gm/pot+ foliar spray with Ridomil @0.25%.
- **T10.** = Soil application of FYM @ 250gm/pot (control).

Discussion

The crop is attacked by several pathogens causing diseases like early blight, late blight, leaf spots, dry rot, charcoal rot, black scurf, common scab, soft rot and viral diseases etc. which are the major constraints in production of potato. Among them late blight is the major diseases throughout the country and in all potato growing country in the world. It also attacks potato tubers in the field, which rot either in the field or while in storage. Late blight may cause total destruction of all plants in a field within a week or two when weather is cool and wet. Even when losses in the field are small, potato may become infected during harvest and may rot in storage. The pathogen has two mating types i.e. A1 and A2. Until 1980s, there was only mating type A1 and reproduce in the absence of its compatible mating type A2, i.e. asexually. Therefore, they do not produce oospores and overwintered only as mycelium in infected potato tubers. Spread of the compatible mating type A2 from Mexico to the rest of the world has made possible the sexual reproduction of oospores in infected above ground and below ground potato tissue. (Agrios, 2005)

[10] Oospore may survive in the soil for 3-4 years. Dormant mycelium in infected tubers causes systemic infection during growth of seedling. After symptom manifestation, large number of sporangia is produced and cause secondary infection. A relative humidity near 100% and temperature between 15 and 25°C are most conductive for disease development. Various methods like cultural practice, chemical, biological and use of resistance varieties are used to manage the disease reported by several workers (Singh, 1996; Joshi and Pundhir, 2013; Shaibala and Pundhir, 2008) [4, 6, 5]. There is no doubt that application of fungicides is a true method for potato protection, but continuous use of fungicides leads to increase in the development resistance strain of Phytophthora infestans (Mont. de Barry) to use both systemic and even protective fungicides. Therefore, search for new, ecofriendly and non-conventional method of plant protection is an indispensable need of these days.

Studied in detail and gave it a generic name *Phytophthora* (plant destroyer) on account of its special feature of indeterminate sympodial sporangiophore with ovoid,

detachable and papillate, sporangia and the fungus got its final title of *Phytophthora infestans* (Mont. de Barry) these observations are in accordance with Anton de Bary (1876) [11].

The earliest symptoms of the disease are often present on lower leaves. Initially pale to dark green spots occur at the leaf tips and margins that change into brown or black lesions later. When lesions on individual leaflets are examined, a zone outside the purplish lesions is found to show a paler than normal green merging with purplish lesion. On the lower side of leaves a white mildew appears on the surface of lesions where the pale and purplish tissues join.

The effect of soil amendment with organic manure and tuber treatment with different bio-formulations on plant height and germination per cent in glass house condition shows that integrated effect were effective in increasing seed tuber germination and vigor of plants (Table-7, Fig-12). Seed tuber treatment with different bio agent was responsible for early breaking of seed tuber dormancy there by increasing the germination per cent of seed tuber.

The observation of germination per cent of tuber was taken at every 24 hours up to 10 days. The data were recorded that the maximum with 100 per cent germination was recorded in T1 treatment is followed by T2 treatment which is found as 72.72 per cent and minimum germination per cent has been

recorded in control is 9.13 per cent. Similarly, plant height was found maximum in treatment T1 with 44.5cm followed by treatment T2, T3, representing value of 43.5cm and 42.5cm, respectively against control which represent a value of 33.4cm (Table-8, Fig-13). The result of experiments conducted by glass house showed that bio-formulation have a stimulatory effect on germination of seeds. Mansoor et al., (2001) [12] reported that the Azotobacter significantly increase the yield characters like tiller and tuber. Kumar et al. (2001) [13] investigated on strains of A. chroococum and their mutants and reported that the strains of A. chroococum better in all the varieties in increased in grain yield over control. Rasool Azarmi et al., (2011) [14] reported that the seed germination rate was affected by Trichoderma application but shoot height and shoot diameter, fresh and dry weight of shoot in tomato seedlings were increased significantly. They also found that soil amended by Trichoderma spp. had marked increase in leaf number and area of leaf. Chandanie et al. (2009) found that, the combination inoculation of Arbuscular mycorrhizal fungi with Trichoderma synergistically increased dry shoot mass when compared with inoculation of Trichoderma and Arbuscular mycorrhizal fungi alone.

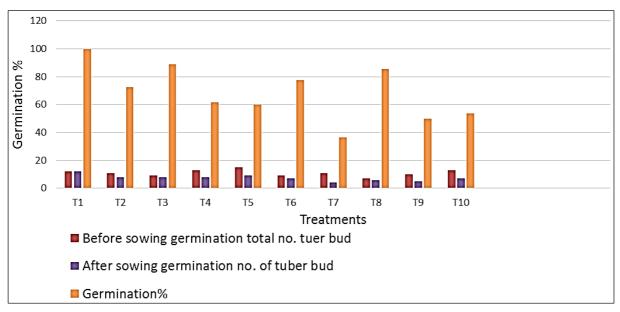


Fig 2: Effect of soil amendment and tuber treatment on germination of seed tuber

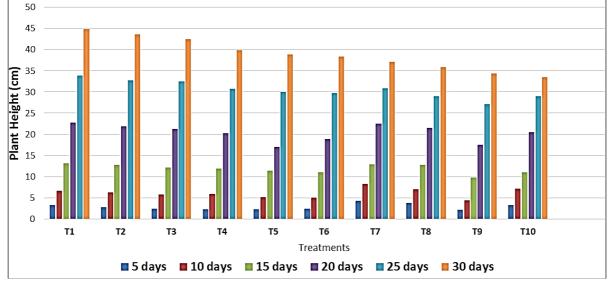


Fig 3: Effect of Integrated Disease Management practices on growth parameters of potato

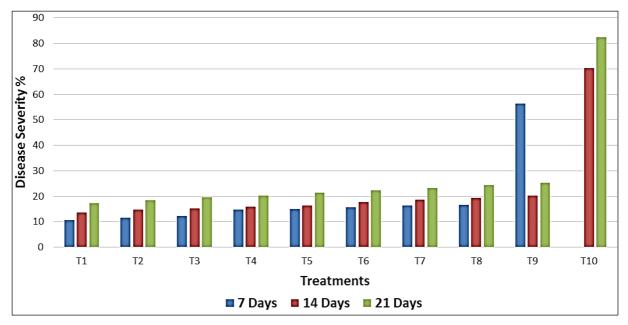


Fig 4: Effect of Integrated Disease Management practices on severity of late blight of potato

That maximum number of large tuber was found in T2 treatment representing with weight of tuber 51.67gm, where treatment was given as soil application of FYM @ 125gm/pot+ mushroom waste with T. harzianum @5gm/kg + tuber treatment with T. harzianum @ 5% + foliar spray with Mancozeb@0.25%. In case of other treatments, there is no large size potato. On the other hand, maximum number of medium size tuber has been found in T2 treatment, representing with weight of tuber 338.33gm. Similarly, maximum number of small size tuber have been found in T3 treatment, the weight of 529.66gm, where treatment was given as soil application of FYM @ 125gm/pot + neem cake@125gm/pot+ tuber treatment with T. viride @5% + foliar spray with Mancozeb @0.25%. Muraj et al., (2017) reported that maximum number of large size tuber and highest vield was recorded from the treatment as given as soil application of FYM @ 150gm/pot + mustard cake @ 150gm/pot + tuber treatment with T. viride + foliar spray with bio formulation of T. viride. Tippannavar et al., (2005) [15] had observed that the Azotobacter significantly increase the tillering, dry matter accumulation and growth parameter. Datnoff et al. (1995) [16] found that Trichoderma spp. enhance the growth of tomato yield. Singh et al., (2015) [20] found that the yield of tomato crop significantly increase by the combine application of seed treatment with T. harzianum + soil application of neem cake powder + foliar spray of carbendazim. Mansoor et al., (2001) [12] also observed that the Azotobacter improved plant height and shoot dry weight significantly.

The effect of integrated approaches on severity of late blight of potato revealed that there is a decline in disease severity due to various treatments (Table-9, Fig-14). The susceptible variety *Kufri Pukhraj* of potato showed a 76.40% severity in case of *P. infestans* treated plants. The minimum late blight severity was recorded in treatment T1 (soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg + tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25%) with value of 9.16% followed by treatment T2 (soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg + tuber treatment with *T. harzianum* @ 5% + foliar spray with Mancozeb@0.25%), treatment T3 (soil application of FYM @ 125gm/pot + neem cake@125gm/pot + tuber treatment

with *T. viride* @5% + foliar spray with Mancozeb @0.25%) and T4 (soil application of FYM @ 125gm/pot + mustard cake@125gm/pot + tuber treatment with *Azotobacter*@5% + foliar spray with Carbendazim @0.05%) with the value of 10.11%, 11.11% and 12.55%, respectively at 7 days age of plant. The decrease in disease severity might be combined activity of soil amendment + tuber treatment + foliar spray of fungicide which stimulate to synthesis of some defense compound in potato against *Phytophthora infestans* (Mont. de Barry).

Similar results have also seen found in case of 14 and 21 days of observation. From the table it is also cleared that the severity continuously increases from 7 to 14 days and 14 to 21 days in all treatments. Nguyen Khanh, et al. (2013) [17] found that the utility of different bio-agents, botanicals and fungicides to manage the early blight of tomato. Biswas et al. (2015) found that seed treatment and soil application with bio fertilizers of Azotobacter declined the disease severity of spot blotch from 73.7% to 42.6% in wheat. Hameedunnnisa Begum (1998), reported that the seed treatment with culture of Azotobacter found effective to check the disease. The effect of dipping seedlings in an Azotobacter suspension was also examined and results showed that fruit yield were highest from seedling treated with Azotobacter. Verma et al (2008) reported that the foliar spray of T. viride (10⁷ CFUs/ml) 24 h before challenge inoculation with the test fungus was found effective in reducing the disease severity under glass house conditions. Yogesh et al. (2015) reported that the minimum disease severity of early blight of tomato with 8.56% was found in case of soil application of FYM + seed treatment with bio formulation of T. harzianum + foliar spray of Mancozeb. Muraj et al. (2016) found that disease severity of late blight was come down from 96.00 to 7.82 per cent due to soil application of FYM and mustard cake + tuber treatment with T. viride + foliar spray with T. viride.

Yield is important parameter for crop production. Increase or decrease of yield determines the profit or loss of any cultivators. Yield parameters depend on number of large size tubers. In the present study revealed that, the highest yield (665.02gm) was recorded from treatment T1(soil application of FYM @ 125gm/pot + mushroom waste with *T. harzianum* @5gm/kg + tuber treatment with *Azotobacter* @ 5% + foliar spray with Ridomil @0.25%) followed by treatment T2 (soil

application of FYM @ 125gm/pot + mushroom waste with T. harzianum @5gm/kg + tuber treatment with T. harzianum @ 5% + foliar spray with Mancozeb@0.25%), and T3 (soil application of FYM @ 125gm/pot + neem cake@125gm/pot+ tuber treatment with T. viride @5% + foliar spray with Mancozeb @0.25%) with the value of 635.33gm, 611.99gm, respectively (Table-10, Fig-15). Kachroo and Razdan (2006) reported that combined application of Azotobacter + Azospirillum with different levels of N significantly increase the grain yield of wheat. Indiresh et al., (2003) [18] found that the response of potato cv. Kufri jyoti to individual and combined inoculation of *Azotobacter* Acetobacter diazotrophicus and Pseudomonas striata and showed significant effect on increasing per cent emergence of tubers, numbers, tuber weight per plant, total tuber yield and

marketable tuber yield. Higher yield was recorded in cauliflower (42.58 t/ha) and cabbage (56.16 t/ha) with bio dynamics package than recommended dose of FYM 23.00 and 22.83 t /ha, respectively. Biswas *et al.*, (2008) observed that seed treatment with *T. harzianum* and foliar spray with fungicides is one of the best strategies to manage brown spot and sheath blight of paddy and also increase yield of crop. Biswas *et al.* (2015) also reported that seed treatment and soil application with bio fertilizers of *Azotobacter* significantly increase grain and straw yield of wheat. Ravindra *et al.*, (2015) [20] reported that seed treatment with *T. harzianum* + soil application of neem cake + foliar spray with carbendazim significantly increased crop yield and decrease disease incidence of Fusarium wilt in tomato.

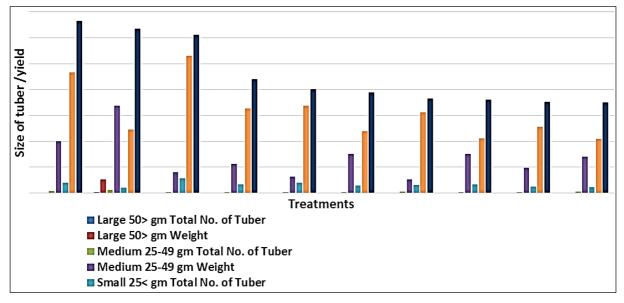


Fig 5: Effect of Integrated Disease Management practices o tuber size and yield of potato

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