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Eco Friendly Management of *Alternaria* sp. causing Leaf Spot of Bitter Gourd (*Momordica charantia* L.)

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A B S T R A C T

Bitter gourd (*Momordica charantia* L.) is one of the most important cucurbitaceous vegetable crops being grown both during warm and rainy season in northern parts of India. It has wide genetic diversity and is grown throughout the tropics and subtropics of the world. But the crop is attacked by a number of diseases such as Anthracnose leaf spot, *Alternaria* leaf blight, *Cercospora* leaf spot, powdery mildew, downy mildew and anthracnose, amongst which leaf spot caused by *Alternaria* spp. is found to cause serious losses throughout Uttar pradesh and other states. A field trail in *Rabi* season in the month of febraury the effect of seed treatment with bio-agents and *Neem* oil were minimize the leaf spot disease severity of bitter gourd. On the basis of single trail it was observed that seed treatment with *Neem* oil @2.5% + *T. viride*@2.5% was most in-effectual against leaf spot disease.

Introduction

Bitter gourd (*Momordica charantia*.L.) is considered to be originated either in Tropical Africa or Indo-Burma region. It is known as Karela in Hindi and Karavellika in Sanskrit. Bitter gourd is one of the most popular vegetable of cucrbitaceae family in the tropical and subtropical countries (Palada and Chang, 2003 and Win *et al.,* 2014). It is widely grown throughout South-east Asian countries including India, China and Nepal (Abascal and Yarnell, 2005 and Raj *et al.,* 2010). It is also grown in Africa, Caribbean and South America as a food and Medicine (Nadkarni, 1993; Warrier *et al.,* 1995; Kumar *et al.,* 2010). Bitter gourd is a popular and demanded vegetable among cucurbits grown in India. India is the second largest producer of vegetables in the world next to china with a production of 181 million tons from an area of 9.575 million hectares with productivity of 17.7 million tons per hectare. In India it is cultivated in an area of 87 thousand million ha

with a production of 917 thousand MT. India shares about 13.4% of the world's vegetable production from about 2.0% of total cropped area (NHB, 2017). In India, the bitter gourd growing states are Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, Maharashtra, Gujarat, Rajasthan, Punjab, West Bengal, Orissa, Assam, Uttar Pradesh and Bihar (Anonymous, 2018).

Bitter gourd is known as balsam pear, bitter melon, bitter cucumber, African cucumber, karela and ampalaya (Behera *et al.,* 2010). The immature fruits are boiled, curried stuffed or sliced and fried, before consumption. The fruits are also pickled, canned and dehydrated. Bitter gourd fruits are good source of carbohydrate, protein, mineral, vitamin and have highest nutritive value among cucurbits (Desai and Musmade, 1998). 100gm of bitter gourd fruit contains 5.6 g protein, 290 mg of calcium, 5mg iron, 5.1mg vitamin A, 170mg vitamin C and 55mg of inorganic phosphorus, small amount of vitamin B complex.

Bitter gourd is commonly attacked by number of diseases such as downy mildew, powdery mildew, gummosis, Phytophtora blight, collar rot, Fusarium wilt, White rot, damping off of seedlings and fungal root rots, root knot nematode, bacterial wilt, bacterial leaf spot, mosaic, watermelon bud necrosis, leaf curl and leaf distortion virus. These diseases are of national importance and cause important economic losses in cucurbits (Mathew and Alice, 2002; Palada and Change, 2003; Rai *et al.,* 2005 and Khan *et al.,* 2014).

Spraying neemarin and neem oil helps in reducing the disease development by washing the leaf surface wet with dew droplets. The presence of the few anti nutrients like saponins, tannins, glycosides, alkaloids, terpenes and flavenoids in the extract of aqueous and oils of *Azardireachta indica* to be responsible in inhibiting mycelia growth of the pathogen. In the castor oil the antifungal activity was due to ricinoleic acids their corresponding salts and oleic acid have been displayed antimicrobial activity (Nieman 1995; Sprong *et al.,* 2001; Skrivanova *et al.,* 2005). It is been assumed that the fatty acids penetrate the lipid membrane and dissociate in the more alkaline interior causing the metabolic uncoupling. In addition, the high inhibition caused by the ricinoleic acid could due to its cytolytic activity by being a solvent of chitin, constituent of the cell membrane of fungi, while the oleic acid has potential antifungal properties attribution to long chain unsaturartion (Agoramoorthy *et al.,* 2007).

Thus, these finding could be significant for an understanding of the basis of the *R. communi s*oil effect on *C. gloeosporioides.*

In clove there is high concentration of the eugenol (83.6%) an antiseptic phenolic compound that can attribute to control the development of microorganism (costa *et al.,* 2011). The major compound is the thyme essential oil are thymol, p-cymene and alpha-terpinene who components have antimicrobial activities (Jakiemiu *et al.,* 2010).

Materials and Methods

Identification of *Alternaria* sp. from infected plant

The typical symptoms of leaf spot observed in the field were small, scattered, circular to oval, yellowish to brown colour spots with concentric rings. Later, several such spots progressed, became irregular and bigger in size. The well developed spots coalesced to form big patches on leaves exhibiting blighted look. Generally, older leaves are infected more severely than younger ones. Keeping in view, the present study was undertaken to aware the management of leaf spot disease in bitter gourd.

Conidiophores dark, mostly simple; determinate or sympodial, rather short or elongate; conidia (porospores) dark, typically with both cross and longitudinal septa; variously shaped, obclavate to elliptical or ovoid, frequently borne acropetally in apical simple or branched appendage; parasitic or saprophytic on plant material (Barnett and Hunter 1998).

Preparation and application of seed treatment:

Neem oil

Neem oil was measured 5 ml with the help of measuring cylinder, was then mixed thoroughly with 100 gm of seeds in a conical flask. The seeds were treated until they were saturated that is around 5-10 minutes (Pratibha *et al.,* 2005). It adhered to the seeds due to sticky nature and sown in the furrow 2 seeds at each point keeping the spacing at 30 cm in between.

Castor oil

Castor oil was measured 5 ml with the help of measuring cylinder, was then mixed thoroughly with 100 gm of seeds. The seeds were treated until they were saturated that is around 5-10 minutes (Rahmatzai *et al.,* 2017).

Clove oil

Clove oil was measured 5 ml with the help of measuring cylinder, was then mixed thoroughly with 100 gm of seeds. The seeds were treated until they were saturated that is around 5-10 minutes (Divyajagan, *et al*., 2018).

Bio-agent, *Trichoderma viride*

Trichoderma sp. was isolated from the soil of the experimental field with the help of serial dilution technique. Culture proliferation was done with the help of single spore technique. Thereafter, with the help of scalpel 5 grams of mycelium was taken and mixed with talc powder. The prepared powder formulation was then used for seed treatment (Ravishankar *et al.,* 2018).

Neem oil + T. viride

Neem oil was measured 2.5 ml with the help of measuring cylinder and 2.5 gm of bio-agent power was measured, then mixed thoroughly with 100 gm of seeds (Gayathri *et al.,* 2018).

Castor oil + T. viride

Clove oil was measured 2.5 ml with the help of measuring cylinder and 2.5 gm of bio-agent power was measured, then mixed thoroughly with 100 gm of seeds.

Clove oil + T. viride

Castor oil was measured 2.5 ml with the help of measuring cylinder and 2.5 gm of bio-agent power was measured, then mixed thoroughly with 100 gm of seeds (Kader *et al.,* 2012).

Disease severity

Per cent disease intensity was recorded at 30, 60 & 90 days after incidence of *Alternaria.* Percentage of Disease severity was calculated in accordance with following formula. The severity of disease was visually assessed in all the plots at weekly interval from first appearance of disease for each treatment. For each plot, the number of infected bitter gourd plants were counted and expressed as a percentage of the total number of bitter gourd plants in that plot. The mean percentage disease severity for each treatment was obtained from the three replications. The data was further statistically analyzed. Disease severity is the percentage of relevant host

tissues or organ covered by symptoms or lesion or damage by the the disease. Severity results from the number and size of the lesions. Disease severity shows about the extent of damage caused by the disease.

Disease severity (%) was calculated by using the following formula:

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease rating}}{\text{Total no. of x maximum rating x disease grade}} \times 100$$

Results and Discussion

Effect of treatments on plant height (cm) of bitter gourd at different DAS interval

Perusal of data (Table 1 and Figure 1) revealed maximum plant height (cm) in Neem oil+ *T. viride* treatment at 30, 60 and 90 days after sowing (121.29, 168.11 and 280.64, respectively),followed by, Castor oil + *T. viride* treatment (116.08, 153.08 and 275.12, respectively),Clove + *T.viride* treatment (112.88,150.21 and 260.60, respectively), *Trichoderma viride* treatment (111.87, 148.05 and 250.95, respectively), Neem oil treatment(108.85,140.22and 235.11, respectively), Clove oil treatment (93.97, 135.15 and 220.09, respectively), Castor oil treatment (88.36,130.68 and 216.30, respectively) and untreated check (77.85, 128.76 and 205.32, respectively). Among the treatments (T2, T7 and T3), (T5, T4,), (T4, T7), (T7, T2,) and (T2, T0) were non-significant to each other. However, all the treatments were significant over control.

Average number of leaves per plant

Perusal of data (Table 2 and Figure 2) revealed maximum average number of leaves/plant in Neem oil + *T. viride* treatment at 30, 60 and 90 days after sowing (68.25, 110.92 and 148.75, respectively), followed by, Castor oil + *T. viride* treatment (59.86, 98.98 and 142.66, respectively), Clove + *T.viride* treatment (58.75, 97.30 and 139.11, respectively), *Trichoderma viride* treatment (58.16, 95.39 and 125.21, respectively), Neem oil treatment (55.62, 88.69 and 120.61, respectively), Clove oil treatment (54.32, 87.49 and 120.08, respectively), Castor oil treatment (52.91, 83.27 and 118.77, respectively) and untreated check (47.36, 73.33 and 109.01, respectively). Among the treatments (T2, T7 and T3), (T5, T4,), (T4, T7), (T7, T2,) and (T2, T0) were non-significant to each other. However, all the treatments were significant over control.

Average number of branches/plant

Perusal of data (Table 3 and Figure 3) revealed maximum average number of branches/plant in Neem oil + *T. viride* treatment at 30, 60 and 90 days after sowing (12.79, 14.00 and 14.71, respectively), followed by, Castor oil + *T. viride* treatment (12.53, 13.03 and 14.01, respectively), Clove + *T.viride* treatment (11.28, 12.16 and 13.31, respectively), *Trichoderma viride* treatment (11.25, 12.11 and 13.21, respectively), Neem oil treatment (10.91, 12.09 and 12.77, respectively), Clove oil treatment (10.14, 11.10 and 12.12, respectively), Castor oil treatment (9.07, 9.77 and 10.38, respectively) and untreated check (7.10, 8.08 and 8.61, respectively). Among the treatments (T2, T7 and T3), (T5, T4,), (T4, T7), (T7, T2,) and (T2, T0) were non-significant to each other. However, all the treatments were significant over control.

First fruit picking

Perusal of data (Table 4 and Figure 4) revealed maximum first fruit picking in Neem

oil + *T. viride* treatment (56.08), followed by, Castor oil + *T. viride* treatment (59.56), Clove + *T.viride* treatment (61.29), *Trichoderma viride* treatment (63.08), Neem oil treatment (65.09), Clove oil treatment (66.29), Castor oil treatment (67.79) and untreated check (70.72). Among the treatments (T2, T7 and T3), (T5, T4,), (T4, T7), (T7, T2,) and (T2, T0) were non-significant to each other. However, all the treatments were significant over control.

Average number of fruits/plant

Perusal of data (Table 4 and Figure 4) revealed maximum average number of fruits/plant in Neem oil + *T. viride* treatment (8.26), followed by, Castor oil + *T. viride* treatment (7.38), Clove + *T.viride* treatment (6.97), *Trichoderma viride* treatment (6.59), Neem oil treatment (6.51), Clove oil treatment (6.04), Castor oil treatment (5.64) and untreated check (5.51).

Among the treatments (T2, T7 and T3), (T5, T4,), (T4, T7), (T7, T2,) and (T2, T0) were non-significant to each other. However, all the treatments were significant over control.

Average length of fruit

Perusal of data (Table 4 and Figure 4) revealed maximum average length of fruits in Neem oil + *T. viride* treatment (18.41), followed by, Castor oil + *T. viride* treatment (16.38), Clove + *T.viride* treatment (15.35), *Trichoderma viride* treatment (14.21), Neem oil treatment (13.27), Clove oil treatment (12.54), Castor oil treatment (12.08) and untreated check (11.05). However, all the treatments were significant over control.

Disease severity of leaf spot in bittergourd

Perusal of data (Table 5 and Figure 5) revealed minimum disease severity in Neem oil + *T. viride* treatment at 30, 60 and 90 days after sowing (7.07, 11.14 and 19.74, respectively), followed by, Castor oil + *T. viride* treatment (8.84, 15.45 and 27.86, respectively), Clove + *T.viride* treatment (9.60, 16.57 and 29.47, respectively), *Trichoderma viride* treatment (12.08, 20.29 and 33.86, respectively), Neem oil treatment (14.78, 22.93 and 45.70, respectively), Clove oil treatment (16.44, 24.16 and 45.99, respectively), Castor oil treatment (18.97, 24.96 and 47.40, respectively) and untreated check (29.05, 52.08 and 70.84, respectively).

Among the treatments (T2, T7 and T3), (T5, T4,), (T4, T7), (T7, T2,) and (T2, T0) were non-significant to each other. However, all the treatments were significant over control.

In the present study result experiment conducted the "Eco-friendly Management of *Alternaria* sp. causing Leaf spot of Bitter gourd (*Momordica charantia.* L)",under the appropriate fruiting carried out in rabi season 2019-2020 to asset the disease severity and growth parameters of crop under field condition.

The experiment was analyzed by using RBD (randomized block design) with three replication in a plot size $2x2m^2$, eight treatments neem oil 5%, castor oil 5%, clove oil 5%, *Trichoderma viride* 5gm\kg, Neem oil 2.5%+ *Trichoderma viride* 2.5%, castor oil 2.5%+*T.viride*2.5%, clove oil 2.5%+ *Trichoderma viride*2.5% along with control.

Table.1 Effect of treatments on plant height (cm) of bitter gourd at 30, 60, and 90 DAS

Treatments		30DAS	60DAS	90DAS
T_0	Control	77.85	128.76	205.32
T_1	Neemoil	108.85	140.22	235.11
T_2	Castoroil	88.36	130.68	216.30
T_3	Cloveoil	93.97	135.15	220.09
T_4	*Trichodermaviride*	111.87	148.05	250.95
T_5	Neem oil + *T. viride*	121.29	168.11	280.64
T_6	Castoroil+*T.viride*	116.08	153.08	275.12
T_7	Clove oil+*T.viride*	112.88	150.21	260.60
S.Ed(±)		**0.60**	**0.59**	**0.42**
C.D.(0.05)		**1.30**	**1.29**	**0.92**

Table.2 Effect of treatments on average number of leaves of bitter gourd at 30, 60 and 90D AS

Treatments		30DAS	60DAS	90DAS
T_0	Control	47.36	73.33	109.01
T_1	Neemoil	55.62	88.69	120.61
T_2	Castoroil	52.91	83.27	118.77
T_3	Cloveoil	54.32	87.49	120.08
T_4	*Trichoderma viride*	58.16	95.39	125.21
T_5	Neemoil+*T.viride*	68.25	110.92	148.75
T_6	Castoroil+*T.viride*	59.86	98.98	142.66
T_7	Cloveoil+*T.viride*	58.75	97.30	139.11
S.Ed(±)		**0.90**	**0.66**	**0.58**
C.D.(0.05)		**1.95**	**1.43**	**1.25**

Table.3 Effect of treatments on average number of branches of bitter gourd at 30, 60and90 DAS

Treatments		30DAS	60DAS	90DAS
T_0	Control	7.10	8.08	8.61
T_1	Neemoil	10.91	12.09	12.77
T_2	Castoroil	9.07	9.77	10.38
T_3	Cloveoil	10.14	11.10	12.12
T_4	*Trichoderma viride*	11.25	12.11	13.21
T_5	Neemoil+*T.viride*	12.79	14.00	14.71
T_6	Castoroil+*T.viride*	12.53	13.03	14.01
T_7	Cloveoil+*T.viride*	11.28	12.16	13.31
S.Ed(±)		**0.51**	**0.36**	**0.28**
C.D.(0.05)		**1.10**	**0.78**	**0.61**

Table.4 Effect of treatments on first picking, average number of fruits/plant and average length of bitter gourd

Treatments		First fruit picking	Average number of fruits/plant	Average length of fruit
T0	Control	70.72	5.51	11.05
T1	Neemoil	65.09	6.51	13.27
T2	Castoroil	67.79	5.64	12.08
T3	Cloveoil	66.29	6.04	12.54
T4	*Trichoderma viride*	63.08	6.59	14.21
T5	Neemoil+*T.viride*	56.08	8.26	18.41
T6	Castoroil+*T.viride*	59.56	7.38	16.38
T7	Cloveoil+*T.viride*	61.29	6.97	15.35
C.D.(0.05)		**1.25**	**0.84**	**0.57**
S.Ed(±)		**0.58**	**0.38**	**0.26**

Table.5 Effect of treatments on Disease severity of leaf spot ofbitter gourd at 30, 60 and 90 DAS

Treatments		30DAS	60DAS	90DAS
T_0	Control	29.05	52.08	70.84
T_1	Neemoil	14.78	22.93	45.70
T_2	Castoroil	18.97	24.96	47.40
T_3	Cloveoil	16.44	24.16	45.99
T_4	*Trichoderma viride*	12.08	20.29	33.86
T_5	Neemoil+*T.viride*	7.07	11.14	19.74
T_6	Castoroil+*T. viride*	8.84	15.45	27.86
T_7	Cloveoil+*T.viride*	9.60	16.57	29.47
S.Ed(±)		**0.43**	**0.56**	**0.60**
C.D.(0.05)		**0.93**	**1.22**	**1.30**

Fig.1 Microscopic view of *Alternaria* sp

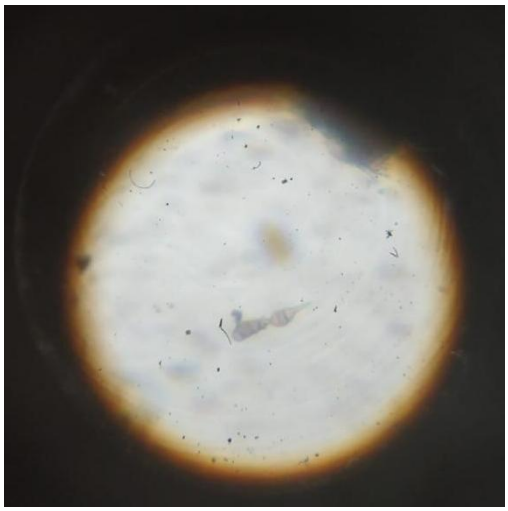


Fig.2 Infected leaf with *Alternaria* leaf spot

Fig.3 Effect of treatments on plant height (cm) of bitter gourd at 30, 60 and 90 DAS

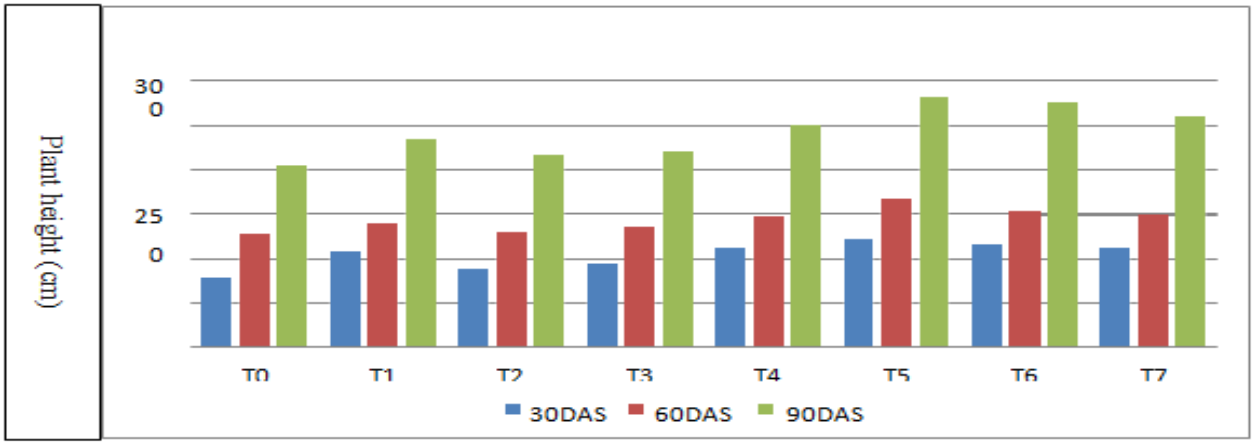


Fig.4 Effect of treatments on average number of leaves/plant of bitter gourd at 30, 60 and 90 DAS.

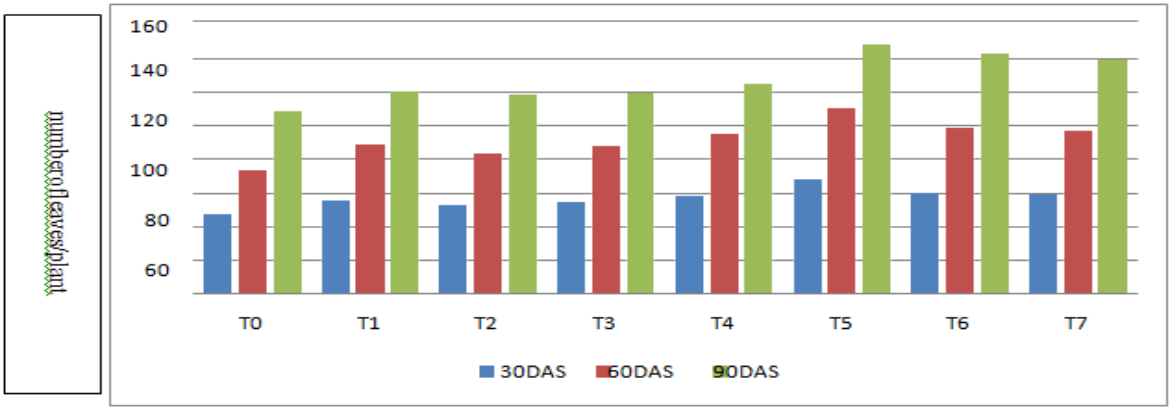


Fig.5 Effect of treatments on average number of branches of bitter gourd at 30, 60 and 90 DAS

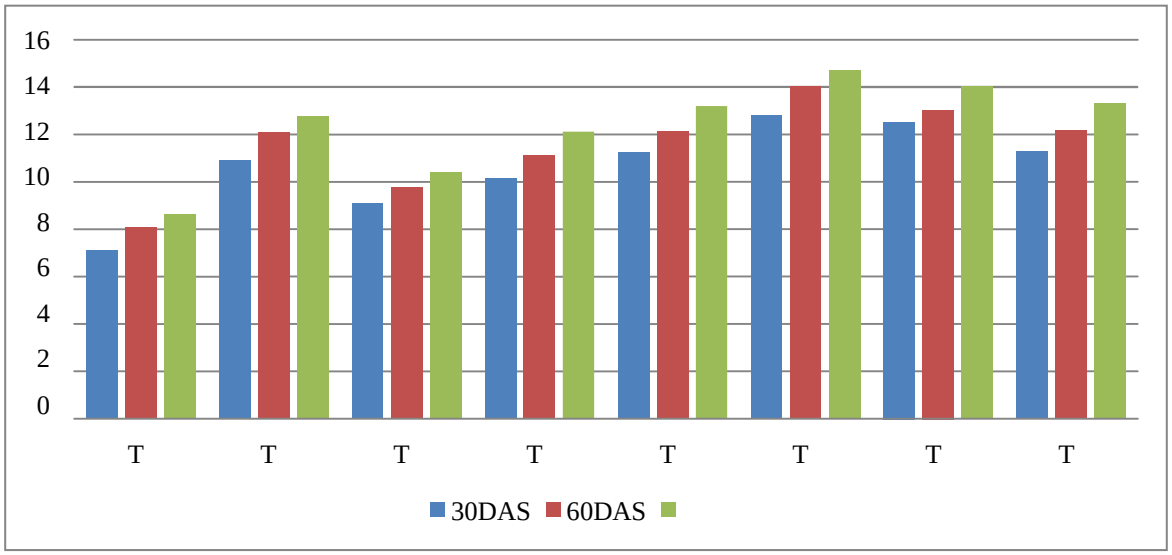


Fig.6 Effect of treatments of Bitter gourd first fruit picking

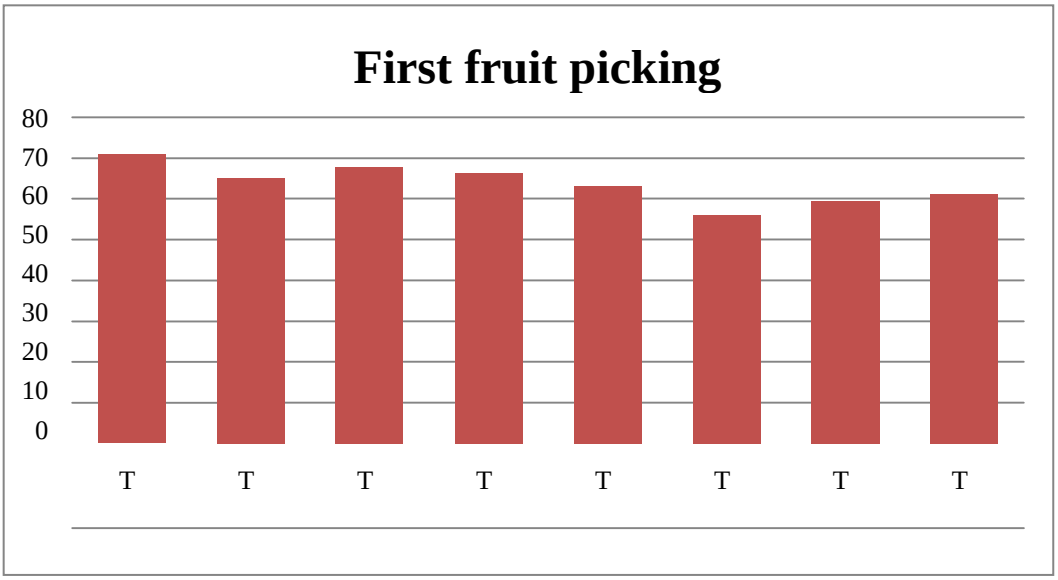


Fig.7 Effect of treatments on average number of fruits per plant of bitter gourd

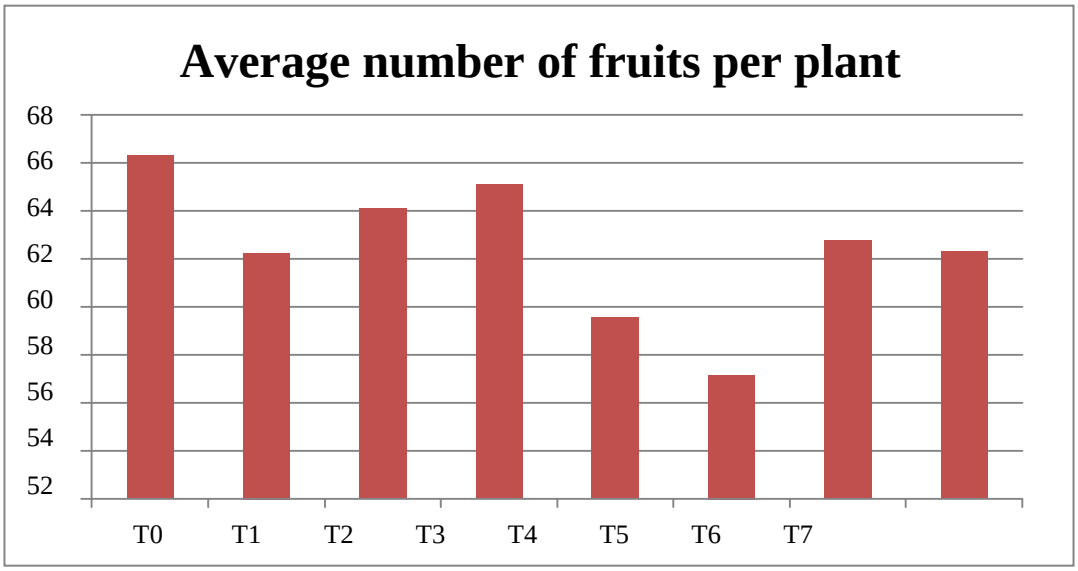


Fig.8 Effect of treatments on average length of fruit of bitter gourd

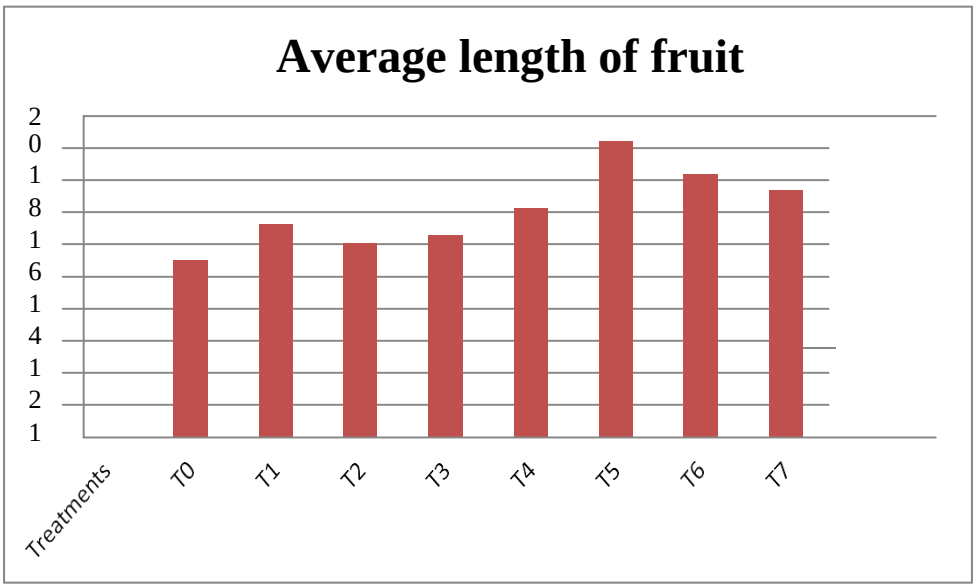
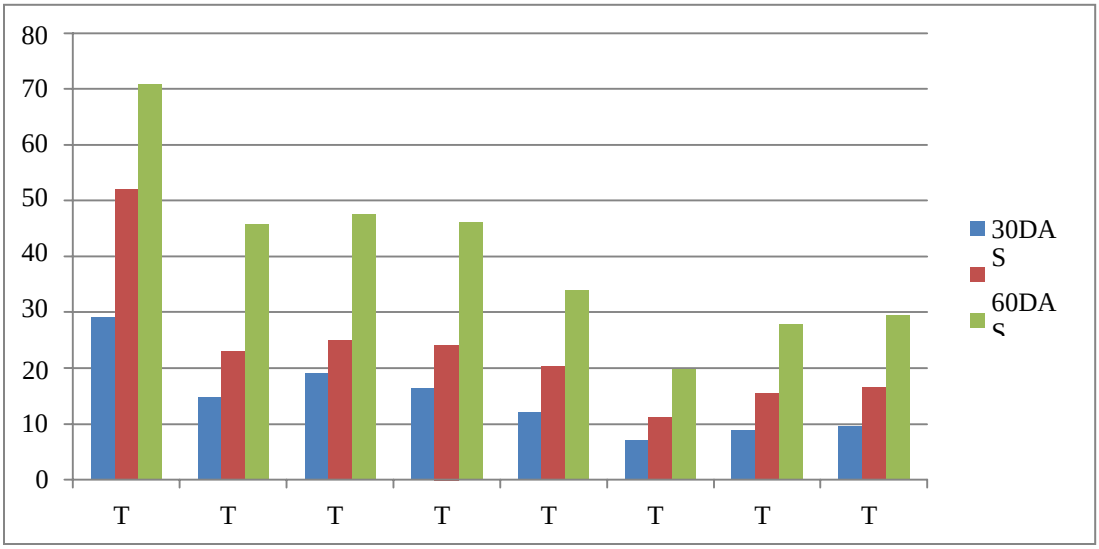


Fig.9 Effect of treatments on Disease severity of leaf spot ofbitter gourd at30, 60 and 90 DAS



Observations were recorded disease severity at 30,60 and 90 DAS(days after sowing) and plant growth parameters such as plant height, number of leaves, number of branches, days to first fruit picking, average length of the fruits, fruits per plant. Experiment revealed that bio-agents with combination of *Trichoderma* increased the growth parameters and it inhibits the disease severity%. Thus, according to experimental findings and results discussed in earlier chapter, it was concluded that bio-agents with combination *Trichoderma* reduced the *Alternaria* leaf spot of bitter gourd.

The concept of eco-friendly management have encouraged the plant protection specialist to go for use of bio-agents for the management of pests and diseases. Also this can avoid the pollution of air, water and soil.

The present study reveals that the bio-agent and neem oil as seed treatment is effectively working on Alternaria leaf spot disease management and has great impact on growth parameters. As the in-organic chemical use on crops is hazards to consumers and they leave the residues in soil which are harmful and loses the fertility of soil. Seed treatment with Neem oil @2.5% + *T. viride* @2.5% recorded minimum disease severity of Alternaria leaf spot and maximum plant growth parameters and yield. So it concluded that the Neem oil @2.5% + *T. viride* @2.5% is best bio resource for management of Alternaria leaf spot and has greater impact on growth parameters and yield. However, the present study was limited to one crop season under Prayagraj conditions, therefore to substantiate the present results more trials are needed for 2-3 seasons for further recommendation.

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