*Int.J.Curr.Microbiol.App.Sci* (2019) *8*(2): 384-391

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 8 Number 02 (2019)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2019.802.043

# Efficiency of Biocontrol Agents, Botanicals and Chemical against *Alternaria porri*

**P.D. Bhandekar, V.S. More* and R.G. Aware**

*Department of Plant Pathology, PGI, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS) – 444104, India*

**Corresponding author:*

**Keywords**

Biocontrol agents, Botanicals, Chemical, *Alternaria porri*

**Article Info**

***Accepted:***
04 January 2019
***Available Online:***
10 February 2019

**A B S T R A C T**

Onion (*Allium cepa* L.) is the important vegetable crop. Under favourable condition onion is attacked by various diseases. Out of which, purple blotch is caused by *Alternaria porri* and is one of the predominant disease of onion. In the present studies, efficacy of bioagents, botanicals and fungicides were tested against *Alternaria porri in vitro* and toxigenic potential of *Alternaria porri.* Among the fungicides, Thiram+Carboxin and Propineb (0.3%) has shown 100% mycelial inhibition of *Alternaria porri*. Maximum mycelial inhibition of *Alternaria porri* was observed with *Ocimum sanctum* (60.83%) followed by Neem leaf extract. Among the bioagents tested, maximum mycelial growth inhibition of *Alternaria porri* was recorded with *Trichoderma viride* (85.45%) followed by *Chaetomium globosum*.

## Introduction

Onion (*Allium cepa* L.) is an important bulb crop of India belonging to family Alliaceae. It is one of the most important winter vegetable crops of India. It is a nutritious vegetable and contains a good amount of Vitamin A and C, rich source of minerals (calcium, manganese and iron) and dietary fibres. It is cool season crop which thrives best in relative cool and moist climate. Purple blotch disease, which is caused by *Alternaria porri* (Ellis) Cif., is one of the most destructive disease restricted to the genus *Allium* and widespread in many regions of the world (Cramer, 2000). Purple blotch disease of onion cause significant reduction in foliar production (Utikar and Padule, 1980) and bulb yield (Gupta and Pathak 1988). The disease is more severe on seed crop as compared to bulb crop sometimes causing a 100% loss of onion seed production (Schwartz 2004, Singh *et al.*, 1992). Under the favourable condition onion is attacked by various diseases. Out of which fungal diseases are main factor responsible for reducing yield. The fungal diseases are, purple blotch (*Alternaria porri*), downy mildew (*Peronospora destructor*), *Stemphylium* blight (*Stemphylium vesicarium*), and basal rot (*Fusarium*

*oxysporium*) (Bollen, 1997; Shivpuri Asha and Gupta, 2011). Among these, purple blotch caused by '*Alternaria porri'* is one of the major diseases of onion. The name purple blotch for this disease was proposed by Nolla (1927). He named the causal organism as *Alternaria allil* which was later amended to *Alternaria porri*. Ajrekar (1920) made first report on leaf spot and blight disease on onion in Bombay and attributed it to *Alternaria* spp. The losses about 50-100% due to purple blotch of onion have been reported by Shahanaz *et al.,* (2007).

The pathogen *Alternaria porri* destructs the leaf tissue which hinders the stimulus for bulb initiation and delay in bulbing and maturation. Sever attack on flowering, onion can completely girdle flower stalks with necrotic tissue, causing their collapse and total loss of seed production capacity (Agale *et al.,* 2014).

The influence of environment on incidence of disease was studied by some workers from different part of countries and reported that high rainfall and high humidity favoured the disease development. *Alternaria porri* on onion occurred following a long period relative humidity > 90% or dew deposition and temperature ranges between 20-25$^0$C (Gupta and Pathak, 1986; Evert and Lacy, 1996).

## Materials and Methods

### Collection of diseased samples

Onion leaves infected with Alternaria purple blotch were collected from the Department of Horticulture, Chilli and Vegetable Research Unit, Dr. P.D.K.V., Akola and farmers field at village Shivapur, Taluka-Barshitakali, District-Akola. Based on symptoms, microscopic examination of diseased samples association of the pathogen as *Alternaria porri* was recorded.

### Source of fungicides, bioagents and botanicals

The fungicides viz., Mancozeb (0.3%), Propineb (0.3%), Copper oxychloride (0.3%), Carbendazim (0.1%), Copper hydroxide (0.3%), Carboxin + Thiram (0.3%), (37.5% + 37.5%) and bioagents, *Trichoderma viride*, *Chaetomium globosum*, *Psudomonas fluorescens* and *Bacillus subtilis*, and aqueous leaf extract of botanicals Pongamia (*Pongamia pinnata*), Bougainvillea (*Bougainvillea spectabilis*), Ocimum (*Ocimum sanctum*), Neem (*Azadirachta indica*) and Mentha (*Mentha arvensis*) (10%) were tested against purple blotch fungi.

### Glassware, plasticware and other materials

Petri plates, glass petri dishes, conical flasks, test tubes, blotter paper and roll paper towel were used in the present studies.

### Method adopted

Potato dextrose agar (PDA) medium was used for isolation and maintenance of cultures.

### Disinfection / sterilization of laboratory materials

To detect the fungi on leaves, the plates were washed with cleaning powder under running water, dried and then disinfected with denatured spirit. However, glass plates were sterilized in hot air oven at 180$^0$ C for 1 hr. before use.

### Isolation of pathogen by tissue isolation method

Infected leaf samples were cut into small pieces with sterilized blade and disinfected with sodium hypochloride (0.2%) solution for two minute. Pieces were washed with three changes of sterilized distilled water and bits

after dried on sterilized filter paper and around flame of spirit lamp were placed on solidified PDA medium in plate. Each plate contained five bits. The plates were incubated at room temperature ($28\pm2^0$C). All these operations were carried out aseptically. The plates were examined regularly. Colonies were developed around the each bit were examined and sub cultured. Based on morphological characters and published literature the fungus was identified as *Alternaria porri*. The pure culture was transferred on PDA slants and maintained for further studies.

## Purification and maintenance of fungal culture

Culture was purified by following hyphal tip method (Vincent, 1947) and culture obtained was maintained on potato dextrose agar (PDA) medium slants at room temperature by adopting subsequent subculturing at periodical, regular intervals. Seven days old culture was used for further studies.

## Pathogenicity test by spray inoculation method

Pathogenicity of fungus was proved by Inoculation of seventeen days old onion seedlings. The fungal suspension was prepared ($4\times 10^4$ spores/ml of water) from seven days old culture and used for inoculation. The seedlings were inoculated by automizing them with fungal suspension. The inoculated and uninoculated seedlings were covered with polyethylene bag for 48 hrs to provide humidity and favourable condition for disease development. Irrigated the seedlings daily to maintain moisture and watched for appearance of disease symptoms on leaves. Chlorotic oval eye shaped lesions were observed after three days of inoculation and full grown purple oval shaped lesion observed after ten days of inoculation. Further the lesion coalesced and spread rapidly on leaf blade and affected leaves showed drying from tip downward after twenty one days of inoculation. Reisolation was made from infected part of inoculated leaves, yielded the same pathogen whereas control plants remained healthy.

## Efficacy of botanicals against *Alternaria porri* by poisoned food method

The poisoned food technique (Nene and Thapliyal, 1993) was employed to evaluate the efficacy of various botanicals against *Alternaria porri*.

## Preparation of aqueous leaf extract of Botanicals

The plant leaves extract were prepared by adopting aqueous extracting method. The standard aqueous leaf extract of the selected botanicals was obtained by grinding the washed plant leaves (100 g) in mortal and pistle in presence of equal amount of sterilized distilled water (100 ml). Prepared leaves extract were filtered through folds of musclin cloth. Colony diameter was recorded in mm and per cent of mycelial inhibition was calculated as per formula given below based on the average of colony diameter. The data of mycelial growth was also subjected to statistical analysis and conclusion were drawn (Vincent, 1947).

$$PI = \frac{C-T}{C} \times 100$$

Where, PI = Percent Inhibition
C = Growth of fungi in control (mm)
T = Growth of fungi in treatment (mm)

**Table.1** Efficacy of fungicides against *Alternaria porri* by poisoned food method

| Sr. No. | Fungicides | Concentration (%) | Mean radial growth(mm) | Mycelial inhibition (%) |
|---|---|---|---|---|
| **1** | Mancozeb | 0.3 | 7.6 | 90.73 |
| **2** | Chlorothalonil | 0.3 | 7.5 | 90.85 |
| **3** | Propineb | 0.3 | 0.0 | 100.00 |
| **4** | Copper oxychloride | 0.3 | 7.3 | 91.01 |
| **5** | Carbendazim | 0.1 | 4.6 | 94.39 |
| **6** | Copper hydroxide | 0.3 | 7.5 | 90.85 |
| **7** | Thiram+Carboxin | 0.3 | 0.0 | 100.00 |
| **8** | Control | - | 82.00 | - |
| | F test | - | Sig. | - |
| | SE(m)± | - | 0.97 | - |
| | CD(P=0.01) | - | 4.04 | - |

**Table.2** Efficacy of botanicals against *Alternaria porri* by poisoned food method

| Sr. No. | Botanicals | Concentration (%) | Mean radial growth (mm) | Mycelial inhibition (%) |
|---|---|---|---|---|
| **1** | Pongamia leaf extract | 10.00 | 38.25 | 56.03 |
| **2** | Bougainvillea leaf extract | 10.00 | 39.53 | 54.56 |
| **3** | Ocimum leaf extract | 10.00 | 34.07 | 60.83 |
| **4** | Neem leaf extract | 10.00 | 36.00 | 58.62 |
| **5** | Mentha leaf extract | 10.00 | 38.54 | 55.70 |
| **6** | Control | - | 87.00 | - |
| | F test | - | Sig. | - |
| | SE(m)± | - | 0.69 | - |
| | CD(P=0.01) | - | 2.05 | - |

**Table.3** Efficacy of biocontrol agents against *Alternaria porri* by dual culture method

| Sr. No. | Bioagents | Mean radial growth (mm) | Mycelial inhibition (%) |
|---|---|---|---|
| **1** | *Trichoderma viride* | 20.75 | 85.45 |
| **2** | *Chaetomium globosum* | 25.50 | 70.68 |
| **3** | *Pseudomonas fluorescens* | 36.75 | 57.75 |
| **4** | *Bacillus subtilis* | 34.50 | 60.34 |
| **5** | Control | 87.00 | - |
| | F test | Sig | - |
| | SE(m)± | 0.53 | - |
| | CD (P=0.01) | 2.78 | - |

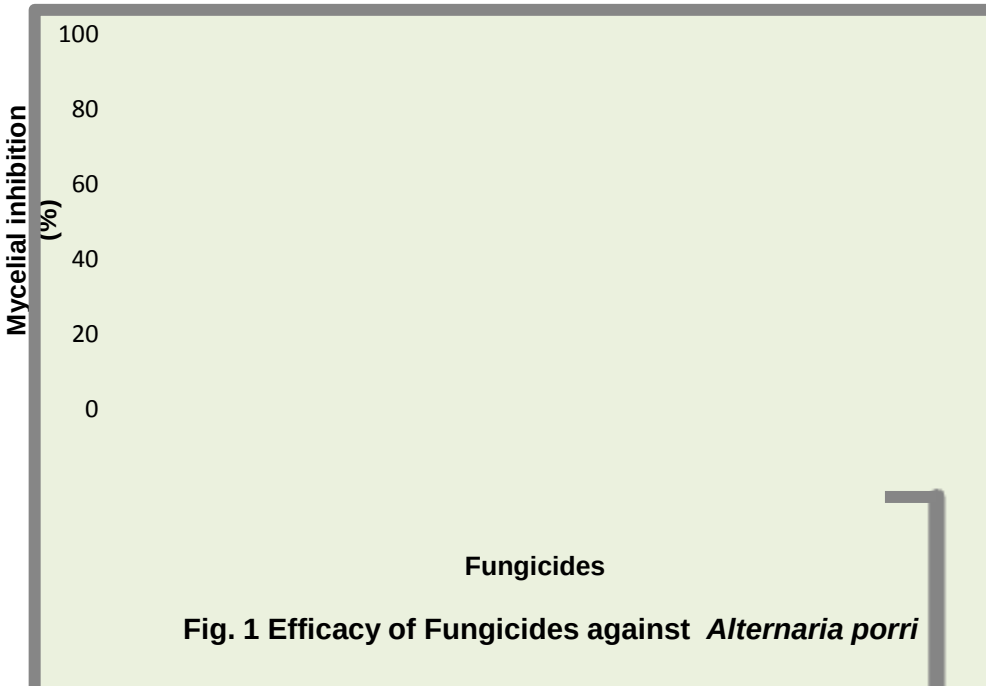


**Fig. 1 Efficacy of Fungicides against *Alternaria porri***

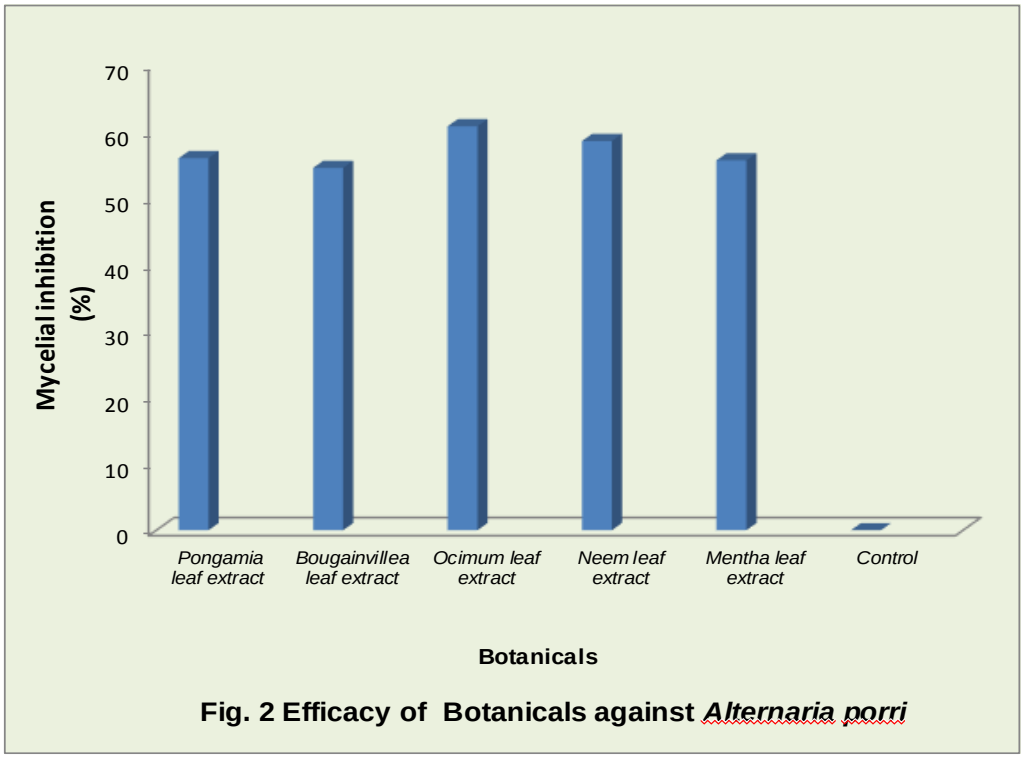


**Fig. 2 Efficacy of Botanicals against *Alternaria porri***

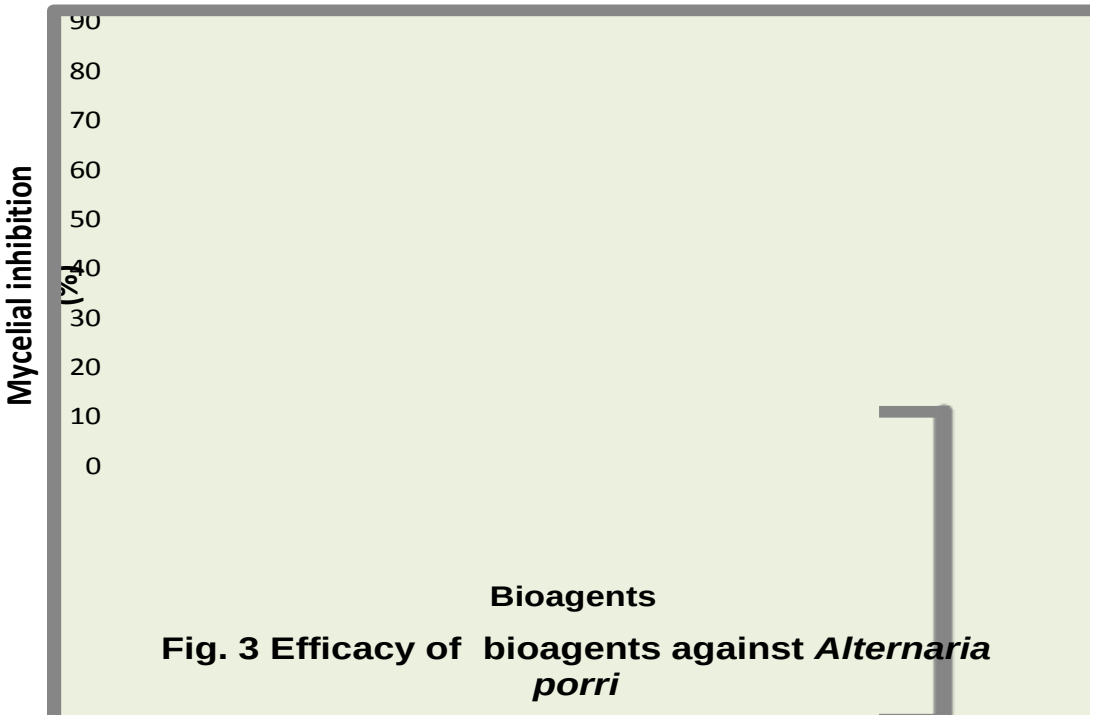


**Fig. 3 Efficacy of bioagents against *Alternaria porri***

## Efficacy of fungicides against *Alternaria porri* by poisoned food method

The results presented in Table 1 indicated that, among all the systemic and non systemic fungicides tested, Propineb (0.3%) and Thiram + Carboxin (0.3%) were found most effective for arresting 100 % mycelial growth of *Alternaria porri*. The present findings of fungicides are in agreement with propineb (Abdel-Hafez *et al.*, 2014, Chetana *et al.*, 2013) against *Alternaria porri*.

Result on the same line has been reported by Pooja Umbarkar (2013) against *Alternaria brassicicola* and *Alternaria raphani* in cauliflower. Ingale (2014) and Kumar *et al.*, (2013) also reported 100 per cent mycelial inhibition against *Colletotrichum capsici* and *Alternaria altenata* in chilli with Thiram+Carboxin and Carboxin (Fig. 1).

## Efficacy of botanicals against *Alternaria porri* by poisoned food method

Among botanicals, the Ocimum leaf extract and Neem leaf extract (10%) were found most effective in arresting maximum mycelial growth of *Alternaria porri* (60.83%) and (58.62%) respectively.

Deepti and Nidhi (2015), Meena (2012) and Ganie *et al.*, (2013) recorded the mycelial inhibition of *Alternaria porri* due to *Ocimum sanctum* by 53.2%, 95.58% and 31.85% respectively. The present result in respect of *Ocimum sanctum* against *Alternaria porri* was also in agreement with Mesta *et al.*, (2007).

The effective role of *Ocimum sanctum* for arresting the mycelial growth (74.7%) of *Alternaria porri* mentioned by the Abdel-Hafez (2014), Thus confirmed the present findings (Table 2 and Fig. 2).

### Efficacy of bioagents against *Alternaria porri* by dual culture method

The fungal bioagent, *Trichoderma viride* recorded maximum mycelial suppression (85.45%) of *Alternaria porri* followed by *Chaetomium globosum* (70.68%), *Pseudomonas fluorescence* and *Bacillus subtilis* (Table 3).

Similar result were recorded by Chethana *et al.,* (2013) Yadav and Mishra, (2013) and Gupta (2012) as they reported maximum mycelial inhibition 79.35%, 94.71% and 53.17% respectively of *A. porri* with *Trichoderma viride* (Fig. 3).

In conclusion, poisoned food method was employed to test the efficacy of various fungicides against *Alternaria porri*, Thiram+ Carboxin (0.3%) and Propineb (0.3%) were found most effective fungicides in arresting (100%) mycelial growth, Carbendazim (0.1%) and Copper oxychloride (0.3%) showed (94.39%) and (91.01%) inhibition of *Alternaria porri* respectively.

Among the five botanicals tested, maximum growth inhibition (60.83%) of *Alternaria porri* was observed with *Ocimum sanctum* by aqueous leaf extract (10%) followed by Neem leaf extract (58.62%) and Mentha leaf extract (55.70%).

The maximum growth inhibition of *Alternaria porri* was recorded with *Trichoderma viride* (85.45%) followed by *Chaetomium globosum* (70.68%) and *Bacillus subtilis* (60.34%) in dual culture method.

Association frequency of *Alternaria porri* was recorded in the range of 40-60% in collected diseased samples.

The *Alternaria porri* was found pathogenic to the onion seedlings causing purple blotch/seedling blight.

Propineb and Thiram+Carboxin was found most effective fungicides against *Alternaria porri*.

Among botanicals, Ocimum and Neem leaf extracts showed maximum mycelial inhibition of *Alternaria porri*.

*Trichoderma viride* was found most effective bioagent against *Alternaria porri* followed by *Chaetomium globosum.*

## References

Abdel-Hafez Sobhy II, Kamal AM Abo-Elysour and Ismall R Abdel-Rahim. 2013. Effects of certain plant extracts to control purple blotch disease of onion plants (*Allium cepa).* J. Plant Physiol Pathol. 1:4.

Abdel-Hafez, S.I.I., Abo-Elyousr, K.A.M., Abdel-Rahim, I.R. 2014. Effectiveness of plant extract to control Purple blotch and S*temphylium* blight of onion. Archives of Phytopathol and Plant Pretection. 47(3): 377-387.

Agale R.C., Kadam J.J., Rite S.C., Kadam J.S., and Pawaskar J.R., 2015. Physiological studies on *Alternaria porri* causing purple blotch of White onion. Journal of Plant Disease Sciences. 9(2): 202-208.

Agale R.C., Kadam JJ, Joshi M.S., Borkar P.G. Symptomology of purple blotch disease of onion and exploitation of fungicides, phytoextract and bioagents against *Alternaria porri*. Species, 2014, 11(31): 63-69.

Ajrekar, S.L., *Ann Rept. Bombay Dept. Agri*., 1920, pp. 121-22; 1921-22, pp. 102-04.

Bollen, G.J. 1997. Slides effect of pesticides on microbial interactions. In: Soil borne plant pathogen (B. Schippers and W. Gams eds.), Academic Press, London, pp: 451-481.

Chetana, B.S., Girija Ganeshan, Archana S.

Rao, and Bellishree, K. 2013. Bioefficacy off botanicals, bioagents and fungicides I management of purple blotch disease of onion. Environment and Ecology 31(23): 947-952.

Cramer C.S. (2000). Breeding and genetics of Fusarium basal rot resistance in onion.- Euphytica, 115(3): 159-166.

Deepti S. and Nidhi D. 2015. Bioefficacy of fungicides and plant extracts against *Alternaria solani* causing early blight of tomato. International Conference on Plant, Marine and Environment Sciences (PMES-2015) Kunal Lumpuri (Malaysia).

Govindrao Ingale, 2014. Seed borne nature of *Colletotrichum capsici* in Chilli and its management. Unpublished M.Sc. Thesis, Department of Plant Pathology, PGI, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Gupta R.B.,L and Pathak V.N., 1988. Yield losses in onions due to purple leaf blotch disease caused by *Alternaria porri*. Phytophylactica 20:21-23.

Gupta R.B.L., and Pathak V.N., 1986. Effect of age host, inoculum density and duration of high relative humidity on development of purple blotch of onion. Phytophylactica 18, 151-152.

Mesta R.K., V.I. Begani, Shrikant Kulkarni and I Shankergoud. 2009. *In vitro* evaluation of fungicides and plant extract against *Alternaria helianthi* causing leaf blight of sunflower. Karnataka J Agr Sci., 22(1): (111-114).

Nene and Thapliyal 1993. Fungicides in Plant Disease Control. Oxford and IBH Publishing Company, Plant diseases, pp. 691.

Nolla, J.A.B. 1927. A new Alternaria disease of onions (*Allium cepa*). Phytopathol. 17:115-137.

Pooja Umbarkar 2013. Alternaria leaf spot of cauliflower and its management. Unpublished M.Sc. Thesis, Department of Plant Pathology, PGI, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Shahanaz, E., Razdan, V.K. and Raina, P.K. 2007. Survival, dispersal and management of foliar blight pathogen of onion. J. Mycol. Pl. Pathol., 37(2): 213-214.

Shivpuri Asha.and Gupta, R.B.L. 2011. Evaluation of different fungicides and plant extract against *Sderotinia selerotiorum* causing stem rot of mustard, Indian phytopath. 54 (20): 272-274.

Utikar P., and Padule D. 1980. A virulent species of Alternaria causing leaf blight of onion. Indian Phytopath. 33(2): 335-166.

Vincent JM (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 59: 850.

**How to cite this article:**

Bhandekar, P.D., V.S. More and Aware, R.G. 2019. Efficiency of Biocontrol Agents, Botanicals and Chemical against *Alternaria porri*. *Int.J.Curr.Microbiol.App.Sci.* 8(02): 384-391. doi: https://doi.org/10.20546/ijcmas.2019.802.043