Int.J.Curr.Microbiol.App.Sci (2022) *11*(01): 293-300

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 01 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1101.035

In vitro Evaluation of Bioagents and Botanicals against *Aspergillus niger* (Van Tieghem) causing Storage Rot of Onion

Mangesh Shivaji Dhumal[1*], J. S. Arekar[2], Y. R. Govekar[1] and P. D. Potphode[3]

[1]*Department of Plant Pathology, DBSKKV, Dapoli, India*
[2]*KVK, Roha, Raigad, India*
[3]*Department of Plant Pathology, COH, Mulde, India*

**Corresponding author*

Keywords

Aspergillus niger, onion, storage rot, bioagents, botanicals, in vitro

Article Info

Received:
08 December 2021
Accepted:
29 December 2021
Available Online:
10 January 2022

A B S T R A C T

In Maharashtra, onion is most important commercial vegetable crop. Storage rot caused by *Aspergillus niger* (Van Tieghem) has been reported as major constraints in successful cultivation and post-harvest management of onion production. Therefore present study was carried out for effective biological management of *Aspergillus niger*. Eight bioagents were evaluated against *A. niger*, among them maximum mycelial growth inhibition was observed in *Trichoderma harzianum* (81.11%) followed by *T. virens* (78.22%), *T. hamatum* (72.66%), *T. viride* (64.88%), *T. asperellum* (61.88%) and *Bacillus subtilis* (57.77%). Lowest mycelial growth inhibition was recorded in *Penicillium* spp. followed by *Pseudomonas fluorenscence* (55.55%). Among all the botanicals studied, complete inhibition of the mycelial growth occurred in *Eucalyptus globules*. This was followed by *Zingiber officinale* (94.44%), *Azadirachta indica* (69.33%), *Ocimum sanctum* (64.88%) and *Curcuma longa* (62.22%). The least (49.67%) mycelial inhibition was recorded with *Lantana camara* followed by *Syzygium aromaticum* (61.11%). Maximum growth was recorded in control.

Introduction

Onion (*Allium cepa* L.) is an important underground bulbous vegetable crop of tropical and sub-tropical countries (Thompson and Kelly, 1979). Red onion is used for domestic consumption and export while the white onion is used mostly for processing (Lawande *et al.*, 2009). The nutritive value of onion varies according to the variety but generally it contains 89.11g moisture, 1.1g protein, 0.1g lipids, 0.4g minerals, 1.7g fiber, 0.34g carbohydrate, 4.24g sugar total, 40Kcal energy, vitamin C and other nutrients in small amounts.

There is a lot of demand for the Indian onions in the world market due excellent pungency of bulbs

compare to other. Onion bulbs suffers from many post-harvest diseases *viz.*, storage rot, bacterial soft rot, green mould, bacterial brown rot which causes considerable losses. Therefore to overcome this losses present study was undertaken to evaluate different bioagents and botanicals under *in vitro* against *A. niger*.

Materials and Methods

Invitro evaluation of bioagents against *Aspergillus niger*

Seven bioagents were evaluated against test fungus by dual culture technique (Dennis and Webster, 1971) in Completely Randomised Design (CRD) with three replications of each treatment.

Treatment Details

Treatments : Eight

Replications : Three

Experimental Design : CRD

For this study, all fungal bio-agents and test pathogen, separately grown on potato dextrose agar medium whereas bacterial antagonist multiplied on NA (Nutrient Agar) media for ten days were used.

The mycelial disc of 5 mm diameter of each antagonist and test fungus were cut with sterilized cork borer and placed aseptically at equidistance exactly on opposite ends of Petri plates containing 20 ml potato dextrose agar medium, whereas bacterial antagonists were streaked at one end of the medium with the help of the sterilized metal loop.

Three replications for each treatment were maintained and the plate with only pathogen at the center, served as control. The plates were incubated at room temperature (28 ± 2°C) and radial growth of the test fungus and the bio-agents was measured by linear measurement. The observations on colony diameter and sporulation of test fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth of test pathogen. The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by following formula (Asalmol *et al.*, 1990).

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

Where,

I = Inhibition per cent

C = Colony diameter (mm) in control plate

T = Colony diameter (mm) in treated plate

In vitro evaluation of botanicals/phytoextracts against *A.niger*

Seven plant species locally available were used to evaluate their efficacy against *Aspergillus niger*.

Experimental Details

Treatments : Seven

Replications : Three

Experimental Designs : CRD

For crude extraction, method used by Sinha and Saxena (1989) were followed with slight modifications. Fresh and healthy plant parts (leaves/cloves/rhizomes) collected from fields were washed with distilled water and air-dried.

Hundred gram required plant parts were crushed in 100 ml of distilled water by W/v method with the help of mortal and pastel. The macerate was filtered through double layered muslin cloth followed by Whatman No.1 filter paper using funnel and filtrate was collected in volumetric flasks of 100 ml capacity. Same procedure were followed for each plant extract. The extract obtained in 100 percent concentration.

To obtain its 10 per cent, 90 ml potato dextrose agar was poured in conical flask (100 ml capacity) and 10 ml of standard plant extract poured in each flask with the help of sterilized pipette and thoroughly mixed. PDA medium amended separately with plant extracts was autoclaved and then poured (20 ml / plate) into sterile Petriplates (90 mm dia.) and allowed to solidify at room temperature. For each treatment, three replications were maintained. Upon solidification of medium, mycelial discs of 5 mm diameter were cut from seven days old culture of *A. niger* with the help of sterilized cork borer. These discs were transferred aseptically to the center of solidified Petri plates of each treatment. Medium devoid of the plant extract served as a control. All these plates were then incubated at room temperature (27 ± 2°C) for growth of fungus. The observations on mean colony diameter of the fungus were recorded when the untreated control plates were fully covered with mycelial growth of the test fungus. The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by the following formula (Asalmol *et al.,* 1990).

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

Where,

I = Inhibition per cent

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Data obtained on per cent growth inhibition were subjected to statistical analysis.

Results and Discussion

Effect of various bioagents on the growth of *A. niger* (Plate-I and Table.3)

Eight antagonists *viz., Trichoderma viride, T. harzianum, T. hamatum, T.virens, T.asperellum, Penicillium* spp., *Pseudomonas fluorescens and Bacillus subtilis* were evaluated *in vitro* for their antagonistic effect against *A. niger* by dual culture method. The observations on mycelial growth inhibition (PGI) recorded when the untreated control plates were fully covered with mycelial growth of the test fungus and are presented in Table 3 (Plate I & Fig. 1). It was observed that all the bioagents significantly inhibited the mycelial growth of *A. niger* over control.

Significantly highest mycelial growth inhibition was recorded in *T. harzianum* (81.11 %) which was at par with *T. virens* (78.22 %) followed by *T. hamatum* (72.66 %), *T. viride* (64.88 %) after seven days of incubation. While average effect on mycelial growth inhibition of *A. niger* was noticed in *T. asperellum* (61.88 %) and *Bacillus subtilis* (57.77 %). Lowest (40.77%) mycelial growth inhibition was observed in *Penicillium spp.* followed by *P. fluorescens* (55.55 %).

The growth inhibition of *A. niger* could be due to fast growing nature of bio-agent, secretion of harmful extra – cellular compounds like antibiotics i.e. gliotoxin and glyoviridin from *Trichoderma spp*. and cell wall degrading enzymes such as glucanases, endochitinases, chitinases and mycoparasitism. The results of present study are in agreement with the results obtained by Lone *et al.,* (2012).

Table.1 List of bioagents evaluated against *A. niger*

Sr. No.	Name of biogents	Sr. No.	Name of biogents
1.	*Trichoderma viride*	6.	*Penicillium spp.*
2.	*T. harzianum*	7.	*Pseudomonas fluorescens*
3.	*T. hamatum*	8.	*Bacillus subtilis*
4.	*T. virens*	9.	Control
5.	*T. asperellum*		

Table.2 List of phytoextracts tested against *A. niger*

Sr. No.	Name of plants	Botanical name of plants	Plant part used	Conc. (%)
1.	Ginger	*Zingiber officinale*	Rhizome	10
2.	Turmeric	*Curcuma longa*	Rhizome	10
3.	Neem	*Azadirachta indica*	Leaves	10
4.	Clove	*Syzygium aromaticum*	Cloves	10
5.	Tulsi	*Ocimum sanctum*	Leaves	10
6.	Nilgiri	*Eucalyptus obliqua*	Oil	10
7.	Ghaneri	*Lantana camara*	Leaves	10
8.	Control	-	-	-

Table.3 Effect of bioagents in growth inhibition of *A.niger*

Sr. No.	Treatments	*Average colony diameter(mm)	Per cent inhibition over control
T_1	*Trichoderma viride*	31.60	64.88 (53.65)
T_2	*T. harzianum*	17.00	81.11 (64.23)
T_3	*T. hamatum*	24.60	72.66 (58.47)
T_4	*T. virens*	19.60	78.22 (62.18)
T_5	*T. asperellum*	34.30	61.88 (51.87)
T_6	*Penicillium* spp.	53.30	40.77 (39.68)
T_7	*Pseudomonas fluorescence*	40.00	55.55 (48.18)
T_8	*Bacillus subtilis*	38.00	57.77 (49.47)
T_9	Control	90.00	-
	S.Em ±	**0.14**	
	C.D at 0.01	**0.57**	

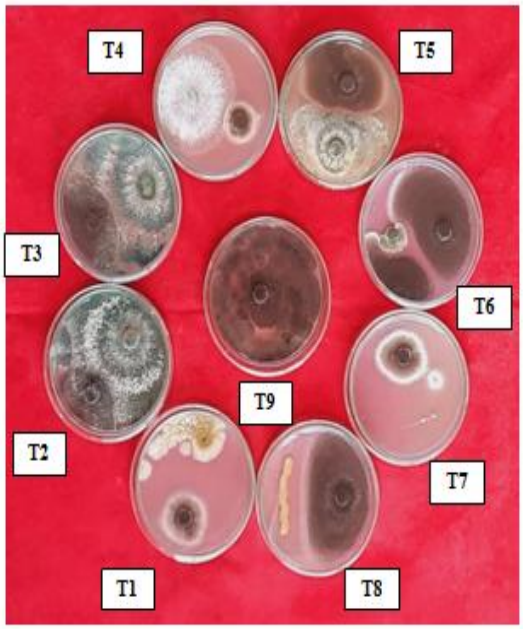
*Mean of three replications. Figures in parenthesis are arcsine transformation.

Table.4 *In vitro* evaluation of phytoextract against *A. niger*

Sr. No.	Plant Name	Scientific Name	Conc.(%)	Mean colony diameter(mm)*	Per cent Inhibition
T_1	Ginger	*Zingiber officinale*	10	05.00	94.44 (76.36)
T_2	Turmeric	*Curcuma longa*	10	34.00	62.22 (52.07)
T_3	Neem	*Azadirachta indica*	10	27.60	69.33 (56.37)
T_4	Clove	*Syzygium aromaticum*	10	35.00	61.11 (51.41)
T_5	Tulsi	*Ocimum sanctum*	10	31.60	64.88 (53.65)
T_6	Nilgiri	*Eucalyptus globules*	10	00.00	100.00 (90)
T_7	Ghaneri	*Lantana camara*	10	45.30	49.67 (44.80)
T_8	Control	-	-	90.00	-
		S.Em ±		**0.08**	
		C.D. at 0.01		**0.33**	

*Mean of three replications. Figures in parenthesis are arcsine transformation.

Plate.1 Effect of bioagents in growth inhibition of *A.niger*



T1 – *Trichoderma viride*

T2 – *T. harzianum*

T3 – *T. hamatum*

T4 – *T. virens*

T5 – *T. asperellum*

T6 – *Penicillium* spp.

T7– *Pseudomonas fluorescence*

T8 – *Bacillus subtilis*

T9 - Control

Plate.2 Effect of phytoextracts on growth inhibition of *A. niger*

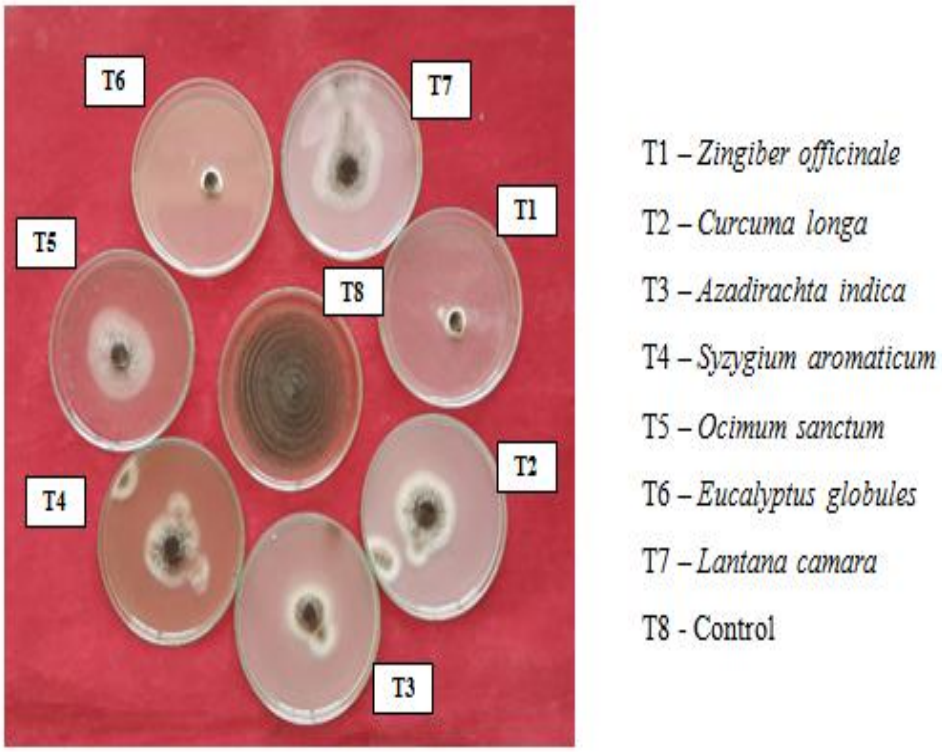


Fig.1 *In vitro* evaluation of bioagents against *A.niger*

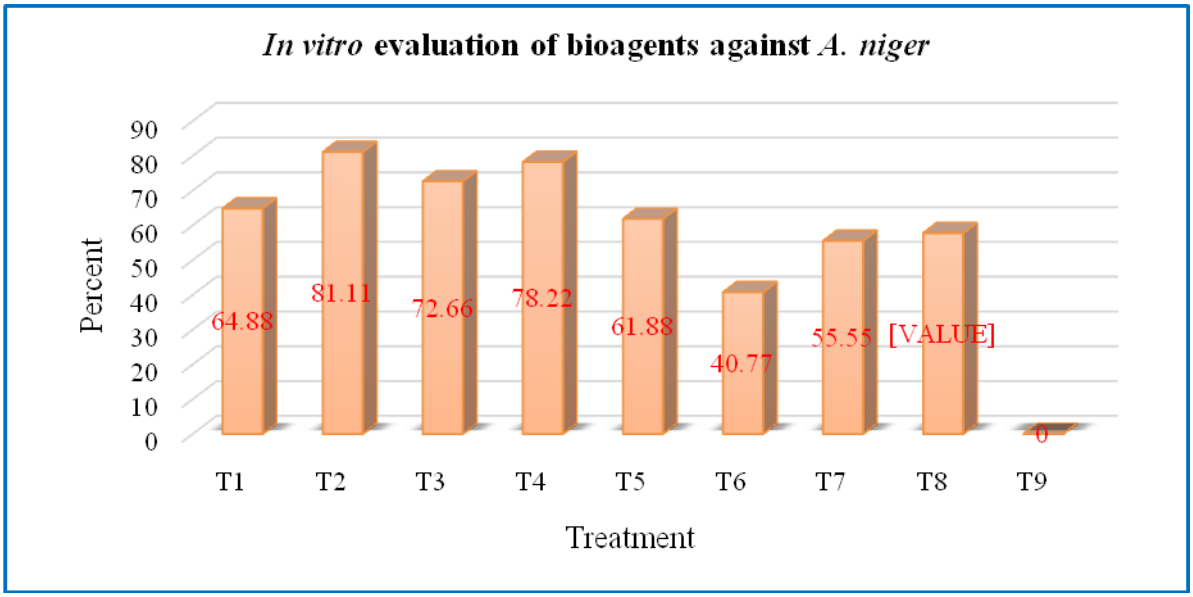
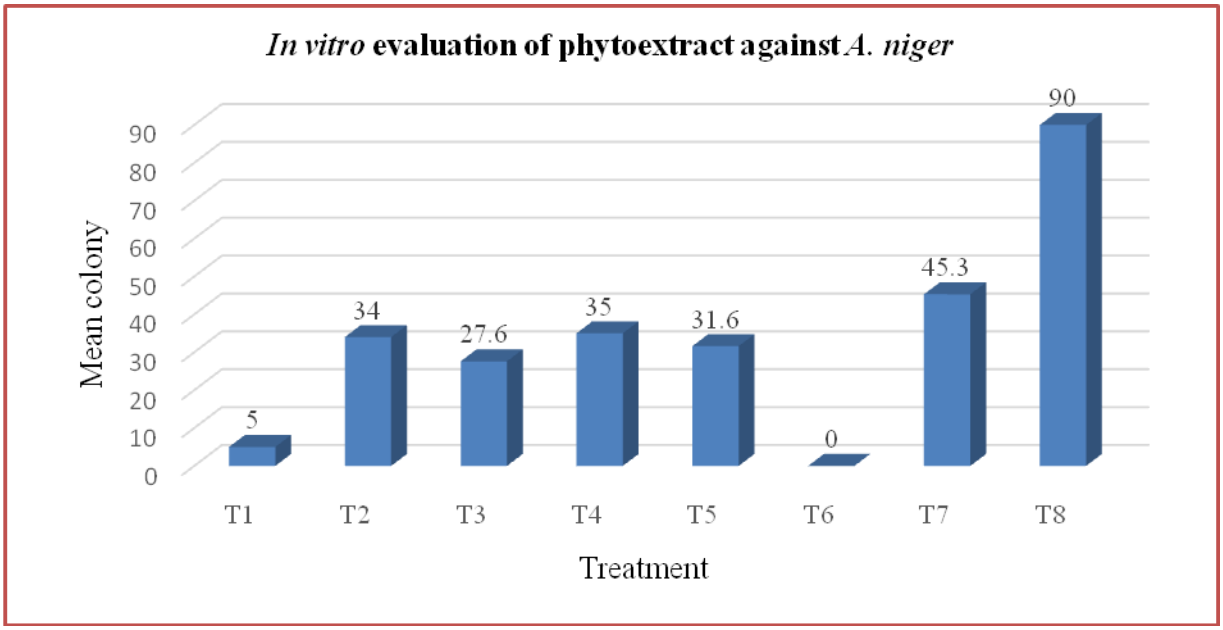


Fig.2 *In vitro* evaluation of phytoextract against *A. niger*



They reported that *Trichoderma harzianum* was the most effective bio-agent against *A.* niger (75% inhibition) in *in vitro*. Gajendra and Vakharia (2012) revealed that, *Trichoderma viride* was more effective (86.2% inhibition) against *A. niger* than *T. harzianum*. (80.4% inhibition). Saranya *et al.*, (2018) reported that *T. viride* isolate T6 significantly reduced the growth (78.63%) of *A. niger* followed by the isolate T9 (75.51%). In comparison to this, *B. subtilis* was less antagonistic (61.27%).

Effect of different phytoextracts on growth inhibition of *A.niger* (Plate-II and Table.4)

The water extracts of seven plant species were studied against *Aspergillus niger* to test their antifungal properties. All the plant extracts were tested at 10 per cent concentration by 'Poisoned food technique'. The plant extracts under study showed antifungal activity against *A.niger*. The data are presented in Table 4 (Plate II& Fig. 2).

All the botanicals tested showed significant inhibition of mycelial growth of *A. niger* at 10 per cent concentration. Cent per cent mycelial growth inhibition of *A. niger* was recorded with *E. globules* followed by *Zingiber officinale* (94.44 %) and *Azadirachta indica* (69.33%). The least (49.67%) mycelial inhibition was recorded with *Lantana camara*.

The results of present study are in agreement with the results obtained by Saranya *et al.*, (2018). They reported mycelial inhibition against test fungus by *Ocimum sanctum* leaf extract (62.97%), *Zingiber officinale* rhizome extract (62.26%) and *Lantana camera* leaf extract (61.59%). Saifeldin *et al.*, (2016) screened the *in vitro* ability of dry bud extract of *Syzygium aromaticum* against soft rot of onion caused by *A. niger* and recorded 96% inhibition in spore germination.

Pudake *et al.*, (2018) studied seven botanicals @10% concentration against *A. niger* and reported eucalyptus oil exhibited 100% inhibition of mycelial growth. They were followed by leaf extract of *O. sanctum* (92.74%), *Azadirachta indica* (76.10%) extract. Futane *et al.*, (2018) evaluated nine botanicals against *A. niger* and it was found that maximum mycelial growth inhibition was recorded with *Allium sativum* (50.99%) followed by *O. sanctum* (29.22%), *A. indica* (23.90%), and least growth inhibition was recorded by *L. camara*, *Z. officinale* as against untreated control

Acknowledgement

Authors are thankful to Professor and Head, Department of Plant Pathology, DBSKKV, Dapoli for providing necessary laboratory facilities and help when required.

References

Asalmol M. N., Sen B and Awasthi J. (1990). Role of temperature and pH in antagonism of *Aspergillus niger* and *Trichoderma viride* against *Fusarium solani*. *Indian Phytopathological Society*., : 11-13.

Dennis, C. and Webster, J. (1971). Antagonistic Properties of Species Groups of Trichoderma III Hyphal Interactions. *Trans. Br. Mycol. Soc*; 57: 363-369.

Futane A. S., Dandnaik B. P., Jadhav P. P. and Salunkhe S. S. (2018). Management of storage diseases of onion by using different botanicals. *Int. J. Curr. Microbiol. App. Sci*. 7(3):3708-3719.

Gajendra H. P. and Vakharia D. N. (2012). Production of lytic enzymes by Trichoderma isolates during in vitro antagonism with *Aspergillus niger*, the causal agent of collar rot of peanut. *Brazilian Journal of Microbiology*., 43(1): 43-52.

Lawande, K. E., Khar, A., Mahajan, V., Srinivas, P. S., Sankar, V. and Singh, R. P. (2009). Onion and garlic research in India. *J. Hortl.Sci*. 4(2):91-119.

Lone, A. M., Wani, R. M., Shaikh, S. A., Sahay, S. and Suliman Dar, M. (2012). Antagonistic potentiality of *Trichoderma harzianum* against *Cladosporium spherospermum*, *Aspergillus niger* and *Fusarium oxysporum*. *J. Bio. Agri. Healthcare*, 2(8): 72-76.

Pudake, S. P., Hingole, D. G., Ghante, P. H., Khaire, P. B. and Swami, C. S. (2018). *In vitro* evaluation of Phyto-extracts and bio-agent against *Aspergillus niger*. *International Journal of Chemical Studies*,7(2): 434-438.

Saifeldin, A. F., Nagerabi, E. L., Awad, H. M., Ahmed, Abdulkadir E. Elshafie (2016). *In vitro* evaluation of selected plant extracts as biocontrol agents against black mould (*Aspergillus niger* Van Tieghem) of onion bulbs (*Allium Cepa* L.) *International Journal of Scientific and Technology Research*, **5**:147-152.

Saranya, R., Anadani, V. B., Akbari, L. F. and Vanthana, M. (2018). Effect of botanicals and bioagents on growth of *Aspergillus niger* (Van Tiegh.) causing black mould in onion. *Int. J. Pure App. Biosci.*, 6(4): 431-436.

Sinha, P. and Saxena, S. K. (1989). Effect of treating tomatoes with leaf extract of certain plant on the development of the fruit rot caused by *Aspergillus niger* in presence of *Drosophila busekii*. *J. Phyto. Res*., 2: 97-101.

Thompson, H. C. and Kelly, W. C. (1979). "Vegetable Crops". Tata McGraw Hill Publishing Company Ltd. Bombay, India, pp: 347-368.

How to cite this article:

Mangesh Shivaji Dhumal, J. S. Arekar, Y. R. Govekar and Potphode, P. D. 2022. *In vitro* Evaluation of Bioagents and Botanicals against *Aspergillus niger* (Van Tieghem) causing Storage Rot of Onion. *Int.J.Curr.Microbiol.App.Sci*. 11(01): 293-300. doi: https://doi.org/10.20546/ijcmas.2022.1101.035