https://doi.org/10.33472/AFJBS.2.4.2020.92-98

ISSN: 2663-2187

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper

Open Access

Antagonistic conflict of *Trichoderma harzianum* against fruit rots pathogens

Udhav Narba Bhale[1]*

[1]Research Laboratory, Department of Botany, Arts, Science and Commerce College, Naldurg Tal., Tuljapur District, Osmanabad - 413601 (M.S.), India. E-mail: unbhale2007@rediffmail.com

Article Info

Volume 2, Issue 4, October 2020

Received : 21 March 2020

Accepted : 25 June 2020

Published : 05 October 2020

doi: 10.33472/AFJBS.2.4.2020.92-98

Abstract

Present work was investigated the study of infected fruits and its pathogens and checked the antagonistic activity with *Trichoderma harzianum. Ficus carica* was to be found highly mycotoxic due to more pathogens followed by *Manilkara zapota* and *Punica granatum* Antagonistic potentials of *T. harzianum* was tested against fruit rots pathogens *in vitro* conditions. Dual culture experiment was conducted for pathogens and *T. harzianum* and data revealed that, the percent inhibition of test fungi proved to be more than 50% antagonism over control. In case of *Fusarium oxysporum* (83.34%) showed highest antagonistic activity followed by others. It was found highly inhibited the radial growth over control. In case of *F. oxysporum*, overgrew beyond 80% (R1-scale) and *Phytophthora parasitica*, *Cladosporim cladosporioides*, *Penicillium* digitatum, *Mucor* sp, *Fusarium* sp and *Geotrichum candidum* overgrew beyond 70% (R2-scale). In case of *Bipolaris cactivora*, overgrow on bioagent (R5-scale) and *Aspergillus flavus, A. tamari* and *Rhizopus nigricans* were found the bioagent and test fungi contact point after inoculation (R4-scale). The results of this study identify as promising biological control agents for further testing against rots disease on fruits.

Keywords: *Fruits, Dual culture, Fruit rot pathogens, Trichoderma harzianum, Antagonistic activity*

1. Introduction

Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. One of the limiting factors that influence the fruits economic value loose due to pathogens attacked. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; and Zhu, 2006). The fungal fruits infection may occur during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly needed to fungal decayed. Present investigation envisages the study of various fungal pathogens responsible for the post-harvest, decay and deterioration of economically important locally available fruits. Thus, many regions there is a clear harvest losses need to investigate the post-harvest diseases (Manoharachary and Rao,

* Corresponding author: Udhav Narba Bhale, Research Laboratory, Department of Botany, Arts, Science and Commerce College, Naldurg Tal, Tuljapur District, Osmanabad - 413601 (M.S.), India. E-mail: unbhale2007@rediffmail.com

1989; and Thakur and Chenulu, 1970). Post-harvest pathogens on some fruits were reported by Basha *et al*. (2009) and Srivastava *et al*. (1964). Fungal spoilage of five selected common fruits were studied and in all 12 spoilage fungi were observed (Madan *et al*., 2017).

Among fungi, *Fusarium* and *Alternaria* usually represent a high mycotoxin in pre-harvested or freshly harvested fruits that are drying, whereas toxigenic species of *Aspergillus* and *Penicillium* represent a higher risk for products in storage and feed processing (Harvey *et al*., 2001; and Hussein and Brasel, 2001). Potential biocontrol agents *Trichoderma* have creator a non-chemical plant disease management system and organic agriculture in particular. *Trichoderma* species has been shown effective against pathogens (Pan *et al*., 2001; and Jash and Pan, 2004). Therefore present attempt was made to screening of antagonistic potential of *T. harzianum* against 13 fruit rots fungi isolated from nine different fruits.

The effectiveness of biocontrol with *Trichoderma* spp. has also been shown by other investigators against *Penicillium digitatum* on citrus fruit (Borras and Aguilar, 1990), *B. cinerea* on grape berries (Elad, 1994), *Monilinia fructigena* on stone fruit (Hong *et al*., 1998), *B. cinerea*, *M. fructigena* and *P. expansum* on apple (Falconi and Mendgen, 1994), and *B. cinerea* and *P. expansum* on yams (*Dioscorea* spp.) (Okigbo and Ikediugwu, 2000).

2. Material and methods

2.1. Study site

The study was investigated in winter season (November -2019 to January-2020) of Naldurg (17.82 °N, 76.30 °E) Osmanabad districts of Maharashtra, India. Naldurg is located at an altitude of 566 m and receives an average annual rainfall of 760 mm.

2.2. Collection of fruits and isolation of pathogens

The infected eight fruits, i.e., *Manilkara zapota L., Ficus carica L., Punica granatum L., Psidium guajava L., Pyrus malus L., Carica papaya L., Citrus reticulata Blanco,* and *Musa paradisiaca L.* were collected from market. Samples were brought in to the laboratory in separate sterilized polythene bags. Rotten samples were kept under refrigeration at 10 °C to prevent further deterioration. Infected fruit parts (1 to 2 mm) were cut into small pieces by sterilized blade then surface sterilized with mercuric chloride (0.1%) for 1 min. The pieces were then washed thrice with sterilized distilled water and dried by sterilized blotting paper. These pieces were placed on Petri dishes (90 mm diameter) containing 20 mL potato dextrose agar (Peeled potato –200 g, Dextrose –20 g, Agar– 20 g and distilled water – 1000 mL, pH – 6.5) (PDA; Sd fine-CHEM Limited Mumbai, India) medium and incubated at 28 ± 2 °C. Nichrome inoculating needles duly sterilized were used to isolate and the pathogens was transferred directly to PDA aseptically and deposited at Department of Botany, Arts, Science and Commerce College, Naldurg.

2.3. Identification of pathogens

The colonies were examined and observed critically under compound microscope. In some cases the infected tissues were directly stained by cotton blue and Lactophenol (Mc Lean and Ivimey, 1965) and observed. The isolated fungi were identified with the aid of standard literature available (Ellis, 1971; Subramanian, 1971; and Barnett, 1960). Pure cultures of the pathogens were maintained in the laboratory on PDA slants for further study. The experiments of this work were carried out in the period 2019 to 2020.

2.4. Dual culture experiment

Antagonistic efficacy of *Trichoderma harzianum* was tested against the isolated fruit rots pathogenic fungi by dual culture experiment (Morton and Stroube, 1955). *T. harzianum* and test fungi were inoculated 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 ± 2 °C for seven days. Monoculture plates of *Trichoderma* served as control. Seven days after incubation (DAI), radial growth of test fungi and *Trichoderma* spp were measured. Colony diameter of test fungi in dual culture plate was observed and compared with control. Percentage of radial growth inhibition (%RGI) was calculated by using the formula: $100 \times [C - T / C]$, Where C = growth in control, and T = growth in treatment (Vincent, 1947).

The degree of antagonism between each of the *Trichodema* species and test pathogens in dual culture was scored on scale of R1-R5 that is, R1=*Trichoderma* completely overgrew pathogens (100% over growth); R2 = *Trichoderma* overgrew at least two-third pathogens (75% over growth); R3 = *Trichoderma* colonizes on one half of the pathogens (50% over growth); R4 = *Trichoderma* and the pathogens contact point after inoculation and R5 = Pathogens overgrow bioagent - *Trichoderma* (Bell *et al*., 1982).

2.5. Statistical analysis

Statistical analysis of the experiments was performed using the *Handbook of Biological Statistics* (Mungikar, 1997).

3. Results and discussion

3.1. Collection of fruits and isolation of pathogens

The infected eight fruits were collected from local market and isolated 13 fruit rot pathogens such as *Aspergillus flavus, Phytophthora parasitica, Fusarium oxysporum, Rhizopus nigricans, Bipolaris cactivora, Cladosporim cladosporioides, Penicillium digitatum, Curvularia lunata, Mucor* sp*, Fusarium* sp*, Aspergillus tamarii, Geotrichum candidum* and *Rhizopus* sp*. Geotrichum candidum. Ficus carica* was to be found highly mycotoxic due to more pathogens followed by *Manilkara zapota* and *Punica granatum* isolates were deposited at Department of Botany, Arts, Science and Commerce College, Naldurg.

3.2. Dual culture experiment

T. harzianum effectively inhibited the radial growth of the fruit rot pathogens. Antagonistic potentials of *T. harzianum* was tested against 13 fruit rots pathogens *in vitro* conditions and revealed the results that of percent inhibition of test fungi proved to be more than 50% antagonistic over control. In case of *Fusarium oxysporum* (83.34%), *Cladosporim cladosporioides* (78%) and *Muor* sp (78%) showed significant antagonistic activity by *T. harzianum* followed by others. *Bipolaris cactivora* (50%) found lowest antagonism by *T. harzianum.* But found highly inhibited the radial growth over control (Table 1, and Figure 1).

Table 1: Antagonistic activity of *Trichoderma harzianum* against fruit rots pathogens

S. No.	Infected fungi	Fruits	CTF	CTH	RGTF (mm)	RGTH (mm)	% Inhibition
1	*Aspergillus flavus*	*Manilkara zapota* L.	89	90	25	65	72.23
2	*Phytophthora parasitica*	*Ficus carica* L.	90	90	28	62	68.89
3	*Fusarium oxysporum*	*Ficus carica* L.	88	90	15	75	83.34
4	*Rhizopus nigricans*	*Punica granatum* L.	90	90	34	56	62.23
5	*Bipolaris cactivora*	*Psidium guajava* L.	90	90	45	45	50.00
6	*Cladosporim cladosporioides*	*Punica granatum* L.	87	90	19	71	78.89
7	*Penicillium digitatum*	*Pyrus malus* L.	88	90	20	70	77.78
8	*Curvularia lunata*	*Manilkara zapota* L.	90	90	26	64	71.12
9	*Mucor* sp	*Ficus carica* L.	90	90	19	71	78.89
10	*Fusarium* sp	*Carica papaya* L.	90	90	25	65	72.23
11	*Aspergillus tamarii*	*Citrus reticulata* Blanco	88	90	24	66	73.34
12	*Geotrichum candidum*	*Musa paradisiaca* L.	87	90	22	68	75.56
13	*Rhizopus* sp.	*Ficus carica* L.	90	90	20	70	77.78
	SE±				2.16	4.65	
	CD (p = 0.05)				4.70	10.14	

Note: Radial growth and % inhibition values are means of three replicates. ± = Standard Error; CTF – Control Test Fungi, CTH – Control *Trichoderma harzianum*, RGTF – Radial Growth Test Fungi, RGTH – Radial growth *Trichoderma harzianum*

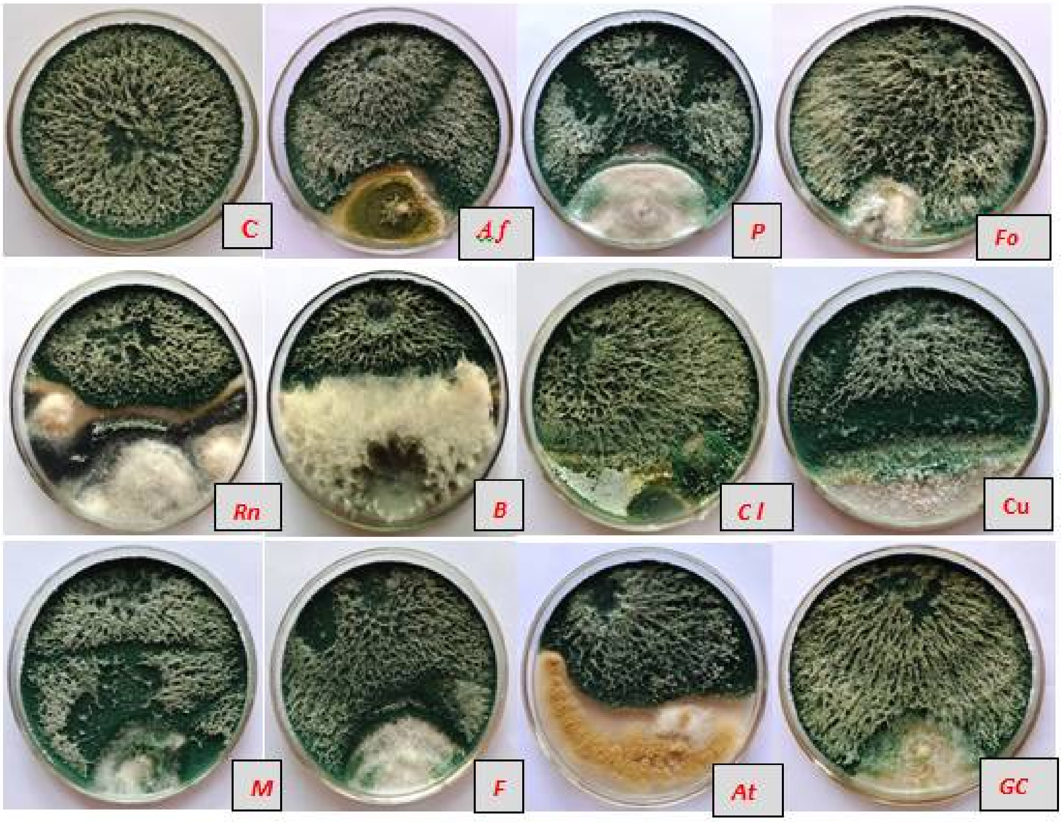

Figure 1: Anatagonistic effects of *Trichoderma harzianum* on fruit rot pathogens

Note: C – Control; Af – *Aspergellus flavus*; P – *Phytophthora*; Fo – *Fusarium oxysporum*; Rn – *Rhizopus nigricans*; B – *Bipolaris*; Cl – *Cladosporium*; Cu – *Curvularia*; M – Mucor; F – *Fusarium*; At – *Aspergillus tamarii*; Gc – *Geotrihum candidum*

According to modified Bell's scale, *F. oxysporum,* overgrew beyond 80% (R1-scale) and *Phytophthora parasitica*, *Cladosporim cladosporioides, Penicillium digitatum, Mucor* sp, *Fusarium* sp and *Geotrichum candidum* overgrew beyond 70% (R2-scale). In case of *Bipolaris* sp, overgrow bioagent (R5-scale) and *Aspergillus flavus, A. tamari* and *Rhizopus nigricans* were found the bioagent and test fungi contact point after inoculation (R4-scale). The results of this study identify as promising biological control agents for further testing against rots disease on fruits. Among the three fruit rot pathogens, only *G. candidum* showed better inhibition by *Trichoderma* species. (Table 2).

Table 2: Evaluation of *Trichoderma harzianum* against fruit rots pathogens by dual culture using Bell's scale*(R)

S. No.	Test fungi	Bell's scale*(R)
1	*Aspergillus flavus*	R4
2	*Phytophthora parasitica*	R2
3	*Fusarium oxysporum*	R1
4	*Rhizopus nigricans*	R4
5	*Bipolaris cactivora*	R5
6	*Cladosporim cladosporioides*	R2
7	*Penicillium digitatum*	R2

Table 2 (Cont.)

S. No.	Test fungi	Bell's scale*(R)
8	*Curvularia lunata*	R2
9	*Mucor* sp	R2
10	*Fusarium* sp	R2
11	*Aspergillus tamarii*	R4
12	*Geotrichum candidum*	R2
13	*Rhizopus* sp.	R3

Note: *Degree of antagonism. R1 = *Trichoderma* completely overgrew pathogens (100% over growth); R2 = *Trichoderma* overgrew at least two-third pathogens (75% over growth); R3 = *Trichoderma* colonizes on one half of the pathogens (50% over growth); R4 = *Trichoderma* and the pathogens contact point after inoculation; R5 = Pathogens overgrow bioagent - *Trichoderma*.

Our results are agreed with previous findings, dual culture of pathogens and *Trichoderma* spp revealed that *T. viride* (Tv-2) (71.41%) highly inhibited the mycelia growth over control (Faheem *et al*., 2010). Seventeen *Trichoderma* strains were screened against *R. solani in vitro*, all strains including *T. harzianum, T. viride* and *T. aureoviride,* inhibited the growth of *R. solani* (Shalini and Kotasthane, 2007). The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda *et al*., 2011). *T. harzianum* was isolated from rambutan orchards in Sri Lanka and proved its antogonistic effect against *Botryodiplodia theobromae* (Sivakumar *et al*., 2000). The results indicated that the treatment with the invert emulsion formulation of *T. harzianum* protected fruit from infection by the primary postharvest pathogens (*Rhizopus stolonifer*, *Botrytis cinerea*, and *Penicillium expansum)* of the fruits (grape, pear, apple, strawberry, and kiwifruit) tested for up to two months and reduced the diameters of decay lesion up to 86% and is a promising treatment to prolong the postharvest shelf-life of fresh fruit (Batta, 2007). It was reported that, *T. harzianum* (54.40%) proved to be more than 50% antagonistic over control in case of *A. niger against fruit rot pathogen on Manilkara zapota* L. (Bhale *et al*., 2013). Sangeetha *et al*. (2009) reported *Colletotrichum musae* cause the postharvest crown rot disease of banana *in vitro* experiments evaluated and *T. harzianum* had performed well when applied singly and the study identified *Trichoderma* antagonists with superior biocontrol potential for the management of the postharvest disease.

4. Conclusion

Trichoderma grows rapidly on a culture medium which should be beneficial during the conflict. Our results concluded that the tested *T. harzianum* reduced the growth of all the tested fruit rots pathogens. *Trichoderma* spp showed significantly reduced the mycelial growth in *F. oxysporum*. We found an inhibition of mycelial growth of the pathogen tested. If there is direct contact between the two fungi, *Trichoderma* sp occupied colonies of fungal isolates. Therefore it can be need for integrated disease management strategy of fruit rot pathogens and safe in transport and storage condition . Hence, *Trichoderma* is an eco-friendly alternative to hazardous postharvest disease as an alternative to pesticides treatments.

Acknowledgment

Authors are thankful to the B.Sc. Graduate students (2019-2020) for carried out this research project.

References

Barnett, H. L. (1960). Illustrated genera of imperfect fungi. Burgress Publishing Company II eds, West Virginia.

Batta, Y. A. (2007). Control of postharvest diseases of fruit with an invert emulsion formulation of *Trichoderma harzianum* Rifai. *Postharvest Biol. Technol*. 43, 143-150.

Basha, R. A., Lal, A. A., Gupta, S., Srivastava, R. and Gupta, P. (2009). Eco friendly management of post harvest fungal pathogen causing crown rot and anthracnose of banana (*Musa paradisiaca*) in Allahabad. *J Mycol Pl Pathol.* 39(1): 14-18.

Bell, D. K., Wells, H. D. and Markham, C. R. (1982). In vitro antagonism of Trichoderma species against six fungal plant pathogens. *Phytopathology*. 72(4), 379-382.

Bhale, U. N., Wagh, P. M. and Rajkonda, J. N. (2013). Antagonistic confrontation of *Trichoderma* spp against fruit rots pathogens on Sapodilla (*Manilkara zapota* L.). *Journal of Yeast and Fungal Research.* 4(1), 5-11.

Borras, D. and Aguilar, R. V. (1990). Biological control of *Penicillium digitatum* on postharvest citrus fruit. *Int. J. Food Microbiol*. 11, 179-184.

Droby, S. (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticul*. 709, 45-51.

Elad, Y. (1994). Biological control of grape gray mold by *Trichoderma harzianum*. *Crop Prot.* 13, 35-38.

Ellis, M. B. (1971). Dematiaceous Hypomycetes. Commonwealth Mycological. Institute, Kew, Surrey, England.

Faheem, A., Razdan, V. K., Mohiddin, F. A., Bhat, K. A. and Saba, B. (2010). Potential of Trichoderma species as biocontrol agents of soil borne fungal propagules. *J. Phytol.,* 2(10), 38-41.

Falconi, J. and Mendgen, K. (1994). Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens: *Botrytis cinerea*, *Monilinia fructigena* and *Penicillium expansum*. *J. Plant Dis. Prot.* 101(477), 38–47.

Harvey, R. B., Edrington, T. S., Kubena, L. F., Rottinghaus, G. E., Turk, J. R., Genovese, K. J. and Nisbet, D. J. (2001). Toxicity of moniliformin from *Fusarium fujikuroi* culture material to growing barrows. *J Food Protect*. 64, 1780-1784.

Hong, C. X., Michailides, T. J. and Holtz, B. A. (1998). Effects of wounding, inoculum density, and biological control agents on postharvest brown rot of stone fruits. *Plant Dis*. 82, 1210-1216.

Hussein, H. S. and Brasel, J. M. (2001).Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology,* 167, 101-134.

Jash, S. and Pan, S. (2004). Evaluation of mutant isolates of *T. harzianum* against R.solani causing seedling blight of green gram. *Ind J Agric Sci*,. 74, 190-193.

Manoharachary, C. and Rao P. R. (1989). Survey and patho physiological studies of fruit rot diseases. Prespectives in plant pathology. Today and Tomorrow's Printers and Publishers, New Delhi.

Mc Lean, R. C. and Ivimey, C. W. R. (1965). Plant Science formule. Macmillan & Co. Ltd, New York.

Morton, D. J. and Stroube, W. H. (1955). Antagonistic and stimulating effects of soil microorganisms upon sclerotium. *Phytopathology*, 45, 417-420.

Mungikar, A. M. (1997). An Introduction to Biometry. Saraswati Printing Press, Aurangabad, pp. 57-63.

Okigbo, R. N. and Ikediugwu, F. E. (2000). Studies on biological control of postharvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride*. *J. Phytopathol*. 148, 351-355.

Pan, S., Roy, A. and Hazra, S. (2001). In vitro variability of biocontrol potential among some isolates of *Gliocladium virens*. *Adv Pl Sci*. 14, 301-303.

Rajkonda, J. N., Sawant, V. S., Ambuse, M. G. and Bhale, U. N. (2011). Inimical potential of *Trichoderma* species against pathogenic fungi. *Plant Sci. Feed.* 1(1), 10-13.

Sangeetha, G., Swaminathan, U. and Muthukumar, A. (2009). Biocontrol with *Trichoderma* species for the management of postharvest crown rot of banana. *Phytopathologia Mediterranea*. 48(2), 214-225.

Madan, S., Sanstuti Pandey., Somdatt, Pandey. and Santosh Kumar, M. (2017). Assessment of fungal spoilage and economic loss of some common fruits at Tehsil - Mauganj, Disst. – Rewa (M.P.). *International Journal of Multidisciplinary Research and Development*, 4(6), 520-522.

Shalini, S. and Kotasthane, A. S. (2007). Parasitism of Rhizoctonia solani by strains of Trichoderma spp. *EJEAF Chem*., 6, 2272-2281.

Sivakumar, D. Wilson, Wijeratnam, R. S., Wijesundera, R. L. C. and Abeyesekere, M. (2000). Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of Rambutan (*Nephelium lappaceum*). *Phytoparasitica*, 28(3), 240-247.

Srivastava, M. P., Sudhirchandra and Tandon, R. N. (1964). Post-harvest diseases of some fruits and vegetables. *Pro. Nat. Acad. Sci. India*. 34(B), 339-342.

Subramanian, C. V. (1971). Hypomycetes: an account of Indian species except cercosporae. Indian Council of Agricultural Research, New Delhi. p. 463.

Thakur, D. P. and Chenulu, V. V. (1970). Chemical control of soft rot of apple & mango fruits caused by *Rhizopus arrhizas*. *Indian Phytopath*. 23, 58-61.

Vincent, J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 150, 850.

Zhu, S. J. (2006). Non-chemical approaches to decay control in postharvest fruit. In: Noureddine, B., Norio, S. (Eds.), *Advances in Postharvest Technologies for Horticultural Crops*. Research Signpost, Trivandrum, India, pp. 297-313.

Cite this article as: Udhav Narba Bhale (2020). Antagonistic conflict of Trichoderma harzianum against fruit rots pathogens. *African Journal of Biological Sciences.* 2(4), 92-98. doi: 10.33472 / AFJBS.2.4.2020.92-98.