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PREVALENCE OF PHYTOPATHOGENIC*ASPERGILLUS NIGER* AND *ASPERGILLUS FLAVUS* ISOLATED FROM VARIOUS SAMPLES IN BANGLADESH

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ABSTRACT

The study was carried out to survey of fungi associated with the determination of fruits, vegetables and betel leaves were collected and two strains of pathogenic fungi- *Aspergillus niger* and *A. flavus* were isolated, identified from 45 collected samples in two different zones (southwestern and central) of Bangladesh. In betel leaves, both isolated fungi were showed their highest percentage of frequency (*A. niger*- 66.66% and *A. flavus*- 46.66%) where *A. niger* showed more pathogenic than *A. flavus*. The result of the pathogenicity test indicates that all the isolated fungi were pathogenic to their respective samples, except orange. The two species of *Aspergillus* spp. were found to be associated as the predominant fungi with the rotten fruits, vegetables and betel leaves.

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INTRODUCTION

The significant source of bacterial and fungal transmission is food, fruits, vegetables and water. Food spoilage refers to several changes which make the food to be toxic and less palatable to consume and these could be associated with alterations in appearance, texture, taste and smell [1]. But raw fruits and some categories of raw vegetables provide various nutrients and possess many health benefits [2]. In the human nutrition supplying, fruits play a vital role in the essential growth factors like as vitamins and essential minerals to the regular diet, which is necessary for the good and healthy consumption of fruits and vegetables products has dramatically increased by more than 30% during the past few decades [3]. Vegetables also provide important roles to combat against cardiovascular and cancerous diseases [4]. Fresh fruits and vegetables are a major source of macro and micro nutrients such as a fiber, minerals and vitamin, thiamin, riboflavin, B-6 niacin, foliate, Vitamin A and E [5]. Fruits and vegetables are well known for their antioxidants compounds that protect against oxidative damage caused by free radicals, and they have been shown to be effective in helping to prevent retinal disease such as muscular degeneration [6].

In Bangladesh, 23.6%-43.5% loss postharvest of fruits and vegetables and over 30% loss of fruits are caused by fungal disease in transit and storage [7][8]. Traditional varieties of fruits like apple, pineapple, grape, orange and tomato are affected by a wide array of microorganisms. The common postharvest and storage fungi of fruits infection are *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Alternaria alternata*, *Fusariumoxy sporum*, *Geotrichum* sp. *Rhizopusnigricans*, *R. stolonifer* and *Penicillium* spp.[9]. Garlic is most herbal medical crops grown all over the world and it's consumed in various forms like as it's known as to reduce blood sugar and cholesterol levels, medicinal pills, powder use in herbal medicine etc. But numerous fungi attack in garlic during storage result in decay causing considerable losses and decreasing the quality. Various fungi were reported to be associated with storage of garlic bulbs such as *A. niger, A. flavus* [10][11]12]. *Aspergillusis* is one of the oldest genera of fungi. It's also one of the most common fungi in agricultural pathogens. *Aspergillus* had become one of the best known and most studied in mould group. *A.niger* can produce a variety of fungal metabolites, termed mycotoxins depending upon growth condition and the strain of the organisms. *A. niger* capable of producing several mycotoxin show ever, mycotoxin

production appears to be controlled by the conditions of fermentation. Another *A. flavus* is a saprophytic and pathogenic fungus. In globally, *A. flavus* is found as a saprophytic in soils and root of a common host of the pathogen on many important agricultural harvests. *A. flavus* has the potential to infect seedlings by sporulation on injured seeds generally excessive moisture conditions and high temp of storage grains and legumes increase the occurrence of *A. flavus* aflotoxin production [13]. Among plant microbial pathogens, fungi are the most important and prevalent pathogens, infecting wild range of host plants, economical losses of crops in the field and harvesting during storage and transportation time. During consumption, Plant based fungal pathogens are unsafe [14]. In betel vine plants are occur some fungal infection like leaf rot disease, foot rot disease and leaf spot disease etc. [15]. Lots of research work has been done of fruits and vegetables rot in fungal infection in others country but insufficient information available in Bangladesh. Therefore, the present investigation was undertaken to find out the selective fungal infection and identify with rotten fruits, vegetables and leaves in Bangladesh.

METHODS AND MATERIALS

Samples sources and preparation : A total 45 samples of *Piper betle* (Betel leaf) (n=15), *Allium sativum* (Garlic) (n=5) and *Solanum lycopersicum* (Tomato) (n=5), *Maluspumila* (Apple) (n=5), *Citrus sinensis* (Orange) (n=5), *Vitisvinifera* (Grape) (n=5) and *Ananascomosus* (Pineapple) (n=5) were collected from two region southwestern zone (Jessore, Khulna and Satkhira) and central Dhaka in Bangladesh. All betel leaf samples were culled in directly betel vine garden (borojes) from South-western part and garlic, tomato, apple, orange, grape, pineapple collected from local market and superstore both central part in Bangladesh. Spots, blights, anthracnose, rots etc. infections are visible in various parts such as stems, leaves, bulbs and fruits in fungi infections. The infected samples were transported in air-tight sterile polyethylene bags and stored in Center of Excellence Laboratory, Department of Microbiology, Primeasia University, Dhaka, Bangladesh at room temperature. Selected samples were examined morphologically, determined their mycoflora and also tested their pathogenicity against selective fugal.

Isolation of Fungal pathogens: Collected infected parts of betel leaves, garlic, tomato, apple, orange, pineapple and grape were washed in tap water for 2-3 minutes and then the infected parts were cut into about 1 cm small pieces, again washed with sterile distilled water in 2-3 times, then were exploitation and dried on the sterilized filter paper (Whatman No: 01) for 10-15 minutes. Then, cutting transmitted portions were placed on the Potato Dextrose Agar (PDA) medium for around two hours to observe proper deposition of fungal spores from the infected parts. Using sterile tweezers, removed the parts from plates and infectious spores were inoculated for 3-7 days in the room temperature [15]. Developing sporulation depends on type of fungi were sub-cultured on fresh PDA medium (with sulfate Streptomycin antibiotics solution) 4-6 times to get pure fungal spore where sulfate Streptomycin antibiotics solution on prevented bacterial contamination in media [16]. Moreover, pure spore of fungus were also placed onto the sterilized filter paper than cut the filter positions were persevered in sterilized vials at freezer on -20°C [17][16]. After the incubation period, the diameter of each inoculum in plates were measured and noted their morphological characteristics and the pure spores identified fungi were persevered as stock culture on half-strength PDA slants in the test tubes.

Identification of fungi: The most significant morphological characteristics of fungus carried out for identification was spore-bearing structure and some fungus body extent characters. The isolated fungi species were identified on the basis of micro and macro morphological structure, identification level of genus using proper media, shape, size, color and arrangement of spore [18]. Isolated pure culture, freshly inoculated to proper media for species identification using macro-morphological and microscopic characteristics were observed [13]. All the isolated fungi were grown in triplicates for the complete isolation and purification of colony and spore morphology. The Frequency of different fungal species was assessed by calculation of the frequency percentage [19] where the values were obtained according to:-

$$\text{Frequency percentage} = \frac{\text{No. of observation in which a species appeared}}{\text{Total number of observations}} \times 100$$

Pathogenecity test of isolated strains based on Koch's Postulate: In Koch's postulates method [20], leaf, stem, body surface tissues cross binding with healthy part to infected of fungus where the isolated fungi were placed into new proper growth media and after inoculation of pure culture replaced into covered moist cotton to promote sporulation of the fungi. Each specimen of isolated fungus were then wrapped to the infected moist cotton covering by healthy parts of selective samples and cotton was kept by adding required water for moist. After 1-7 days inoculated plant were keenly observed, recorded of typical symptoms produced by the pathogen and re-isolation and identification of fungi were done [20].

RESULTS AND DISCUSSION

In present study, the target pure cultures of the isolated fungi were identified according to the cultural properties, morphological, and microscopic characteristics of fungi. A total of 45 samples (leaves, fruits and vegetables) spoilage molds were Isolation from two specific fungi, *A. niger* [18] and *A. flavus* [15], were deteriorating betel leaves, garlic, tomato, apple, pineapple and grape from collected samples but orange did not find target fungal activity (Table 1, Figure 1).

Table 1. Isolated and identification of fungi from various sources

Name of Samples	Number of sample	*Aspergillus niger*	*Aspergillus flavus*
Betel Leaves (paan)	15	10	7
Tomato	5	0	2
Garlic	5	3	0
Apple	5	3	2
Orange	5	0	0
Garpe	5	2	2
Pineapple	5	0	2

In betel leaves, both fungi, *A. flavus* and*A. niger*, were isolated and their percentage of frequency 66.66% and 46.66%, respectively. *A. flavus* were identified in tomato, apple, grape and pineapple and three of them showed the similar percentage of frequency 40% against *A. flavus* and garlic (60%), apple (60%) and grape (40%) of frequency showed against *A. niger* (Figure 2 and 3). Identified *A. niger* and *A. flavus* from selective samples was subjected to pathogenicity test by Koch's Postulates.

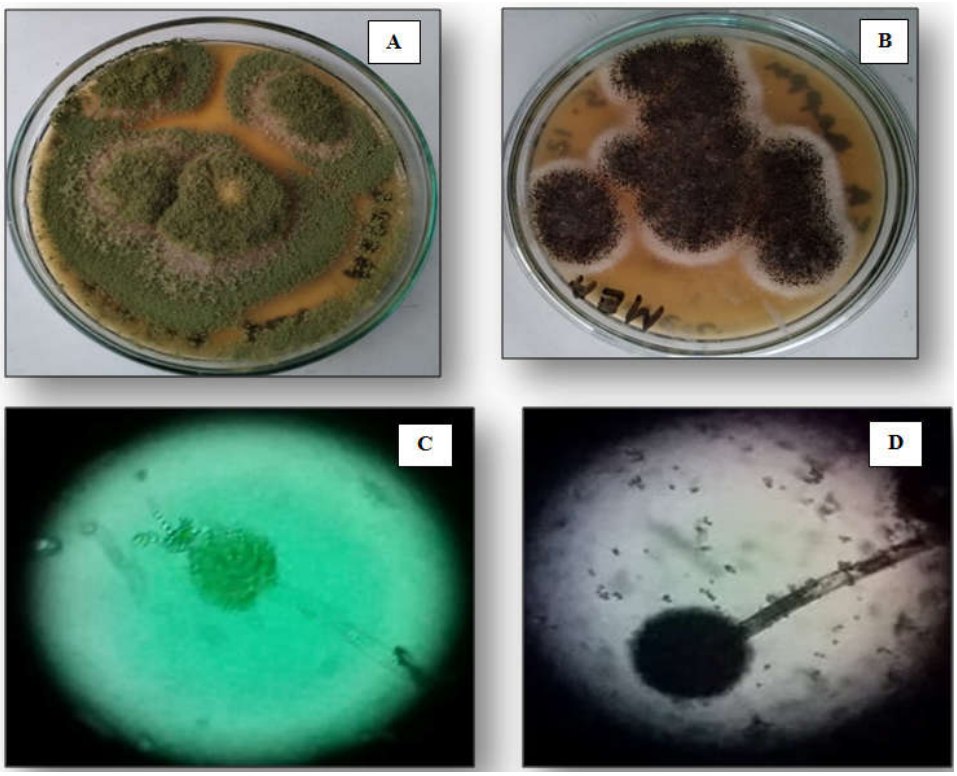

Figure 1. Isolated fungi on PDA plates (A:*A. flavus*, B: *A.niger*) and Microscopic structure of isolated fungi and spores appearance(C: *A. flavus;* D: *A. niger)*

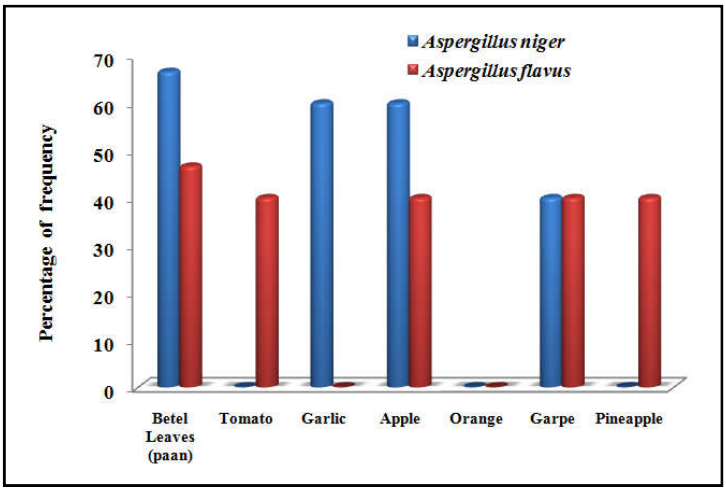

Figure 2. Frequency test of both identified fungus

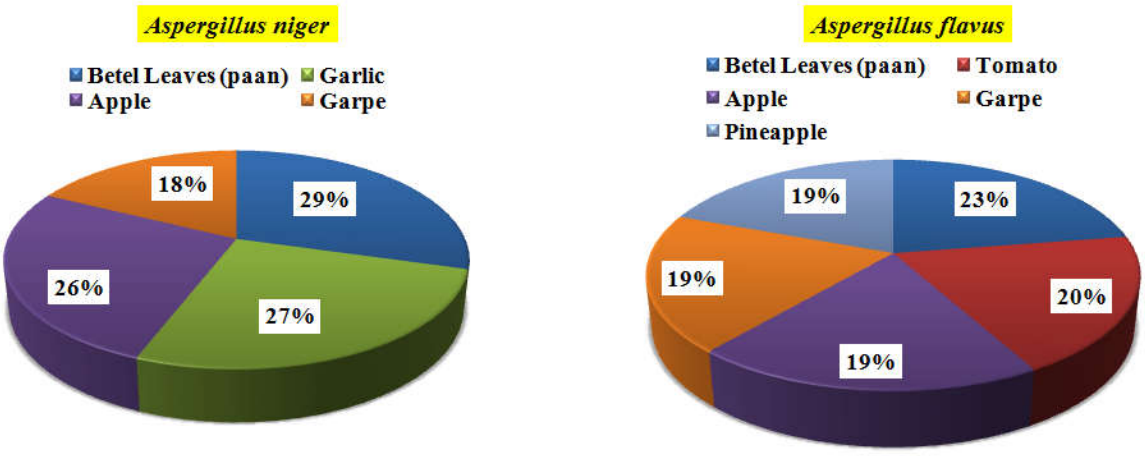

Figure 3. Total percentage of identified fungus against selected samples

Table 2. Pathogenicity testby Koch's postulation

Isolates	Observation						Mortality (%)	
	Mycelium formation (days)		Wilting (days)		Killing (days)			
	A. niger	*A. flavus*	*A. niger*	*A. flavus*	*A. niger*	*A. flavus*	*A. niger*	*A. flavus*
Betel leaves	2	2	3	3	4	4	100	100
Garlic	3	2	4	3	5	5	100	100
Tomato	2	2	3	3	4	4	100	100
Apple	3	4	5	6	6	7	100	100
Graph	2	2	3	3	4	4	100	100
Pineapple	4	4	6	6	8	9	100	100

Take on each healthy sample to inoculate the isolated fungus to exhibit to typical symptoms. After 2-4 days selected samples showed mycelium formation growth on their skin, bulk and their plant base. On the 3-6 days of inoculation, spread of infections rapidly towards the isolated place of the inoculation and started to germinate producing. Finally, the artificially inoculated samples developed by characteristics symptoms resulting in healthy selective samples (Table 2). All samples have done twice the pathogenicity test. On re-isolation from the artificially inoculated fungus from selective samples were found that the pathogen represented same characteristics on PDA culture as found earlier on isolation from naturally infected selective samples caused by *A. niger* and *A. flavus.* Most of the selected samples in these studies showed *Aspergillus* infection except orange could not show infection. Betel leaves, apple and grape samples identified both *Aspergillus* species (*niger* and *flavus*). In the previous study resulted as a similar result in the present researcher. Bashar *et al*. (2012), rotten fruits infection (apple and grape) in Dhaka city showed *A. niger* was the highest frequency of 44% in apple and 11.33% *A. flavus* in the grape as similar as our study also showed *A. niger* 60% in apple and *A. flavus* 40% in grape [7]. *A. flavus* was responsible of pineapple infection; in pervious study also showed similar result in pineapple infection [21]. *A. niger* is most known as the Black mould. *A. flavus* and *A. niger* also parasitizes man and animals and also cause a number of disease grouped under the name Aspergillosis. Without getting sick, most of the people breathe *Aspergillus* spores in their daily life. But, *Aspergillus* infection due to a higher risk of developing health problems that have weakened immune systems or lung diseases infection and allergic reaction.

This *Aspergillus* infection may also be seen in the human ear and is called Otomycosis. *A. flavus* is reported to produce the mycotoxin known as Aflatoxin which is a significant potent carcinogenic, toxigenic, mutagenic, teratogenic and has been directly correlated with adverse health effects, such as liver cancer and other organs disease in many animal species [22]. In the present study; tomato showed *A. flavus* (20%) infection that was most similar result to show in previously in Nigeria [23]. *A. niger* was found in black mold garlic in our study that was most common in other research. Most of the research showed infection of garlic bulbs decay yielding the associated of one or more fungi but *Fusarium solani*, *Botrytis allii* and *Aspergillus niger* were the most common fungi associated with rotted garlic [24][25]. In our study *A. niger* showed in 27% in garlic isolation in total percentage of *A. niger*. In this study, Betel leaves showed the highest percentage of infection in *A. niger* (29%) and *A. flavus* (23%). In previous study, showed betel leaves infected in *Aspergillus* spp. (5.43%) in highest fungal from that research [26]. Another research reported that the stem root and yellow leaf in betel leaves disease were caused by *Aspergillus* spp. [15].

Betel leaves are chewed directly human; so there is need to evaluate the fungicide reduce in leaves before the human consumption. It's difficult to gain control of diseases at the same time where both fungal and bacterial pathogens are associated. But chemical fungicide application is effective method of plant disease control is indisputably. However, indiscriminate use fungicides could produce environmental and health hazards especially direct consume fruits, vegetables and leaves (Betel leaves). *Aspergillus* spp. is one of the most common fungi sources in agricultural different plant parts (seed, root, leaf, and stem) occur infection is caused or do the harmful effect such reduction in crop yields, loss in germination percentage, development of plant disease discoloration and shriveling, biochemical change in a seed. For that reason, the present finding in this study focused on *A. niger* and *A. flavus* were isolated from Betel leaves, vegetables and fruits in the southern and central part of Bangladesh. The high prevalence of fungi isolates in the selected fruits and vegetables showed that fungi are the major cause of the spoilage. Deterioration of most fruits, vegetables and leaves is caused by fungi infection. The isolated fungi are economic and public health importance. Isolated fungi *A. niger* have been reported to produce mycotoxins that can be harmful to human and animals and also harmful aflatoxin produce by *A. flavus*. For those reasons, we recommend using fungicides spraying timely of the harvest to reduce the damaging activities of the fungal pathogens and contamination with mycotoxins and related fungal metabolites infection that might be hazardous to human health.

REFERENCES

Akinmusire OO, "Fungal species associated with the spoilage of some edible fruits in Maiduguri northern eastern Nigeria," *Adv. Environ. Biol.*, 5(1): 157–161, 2011.

Ofuase MWO, "Microorganisms responsible for the spoilage of tomato fruits, Lycopersicum esculentum, sold in markets in Benin City, southern Nigeria," *Sch. Acad. J. Biosci. Sch. Acad. J. Biosci*, 2(7): 2321–6883, 2014, [Online]. Available: www.saspublisher.com.

Barth M, Hankinson TR, Zhuang H and Breidt F, *Compendium of the Microbiological Spoilage of Foods and Beverages*. Springer, New York, NY, 2009.

Akter R, Tanu NI and Uddin MA, "Isolation and quantification of bacterial isolates from citrus fruits and determination of their anti-bacterial activity against selected pathogens," *Stamford J. Microbiol.*, 3(1): 30–33, 2015, doi: 10.3329/sjm.v3i1.22750.

Rickman JC, Barrett DM and Christine MB, "Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds," *J. Sci. Food Agric.*, 87(2007): 930–944, 2007, doi: 10.1002/jsfa.

Wada L and Ou B, "Antioxidant activity and phenolic content of Oregon caneberries," *J. Agric. Food Chem.*, 50(12): 3495–3500, 2002, doi: 10.1021/jf011405l.

Bashar MA, Shamsi S and Hossain M, "Fungiassociatedwith rottenfruits in Dhaka Metropolis," *Bangladesh J. Bot.*, 41(1): 115–117, 2012, [Online]. Available: https://doi.org/10.3329/bjb.v41i1.11090.

Hassan MK, Chowdhury BLD and Akhter N, "Post Harvest Loss Assessment : A Study to Formulate Policy for Loss Reduction of Fruits and Vegetables and Socioeconomic Uplift of the Stakeholders," Bangladesh, 2010. [Online]. Available: http://fpmu.gov.bd/agridrupal/content/post-harvest-loss-assessment-study-formulate-policy-loss-reduction-fruits-and-vegetables-and.

Bhale UN, "Survey of market storage diseases of some important fruits of Osmannabad District (M.S.) India," *Sci. Res. Report.*, 1(2): 88–91, 2011, [Online]. Available: https://jsrr.net/Volume1September2011.html.

Ghangaonkar NM., "Incidence of Mycoflora on Garlic (Allium Sativum L .) Bulbs," *Int. Res. J. Biol. Sci.*, 2(7): 64–66, 2013, [Online]. Available: http://www.isca.in/IJBS/Archive/v2/i7/13.ISCA-IRJBS-2013-096.php.

Prasad BK, Thakur SP, Shankar U and Kumar S, "Decay of garlic bulb in the field: a new disease report," *Indian Phytopathol.*, 39(4): 622–624, 1986.

Mathur RL and Mathur BL, "Black mould of garlic (Allium sativum L.)," *Sci. Cult*, 23: 172–173, 1958.

Barwant M and Lavhate N, "Isolation and maitenance offungalpathogens *Aspergillus niger* and *Aspergillus flavus*," *Int. J. Appl. Nat. Sci.*, 9(3): 47–52, 2020.

Riddhi MP and Yogesh TJ, "Evaluation of Fungitoxic Potency of Piper Betel L.(Mysore Variety) Leaf Extracts Against Eleven Phyto," *Cibtech J. Bio-Protocols*, 2(2):, 21–28, 2013.

San SM and Naing Z, "Isolation of Pathogenic Fungi from Piper betle L .," *Univ. Mandalay OA Repos.*, 2016, [Online]. Available: http://umoar.mu.edu.mm/handle/123456789/41.

Motlagh MRS and Kaviani B, "Characterization of new *Bipolaris* spp.: The causal agent of rice brown spot disease in the north of Iran," *Int. J. Agric. Biol.*, 10(6): 638–642, 2008.

Motlagh MRS, "Isolation and characterization of some important fungi from echinochloa spp. the potential agents to control rice weeds," *Aust. J. Crop Sci.*, 4(6): 457–460, 2010.

Barnett HL and Hunter BB, *Illustrated Genera of Imperfect Fungi 4th Edition*. 1998.

Giridhar SM and Ready P, "Incidences of mycotoxin producers on spices from Andhra Pradesh," *J. Indian Bot. Soc.*, 76:161–164, 1997.

Jahan A, Islam MR, Rahman MM, Rashid MH and Adan MJ, "Investigation on foot and root rot of betel vine (Piper betle L.) in Kushtia district of Bangladesh," *J. Biosci. Agric. Res.*, 7(1): 590–599, 2016, doi: 10.18801/jbar.070116.71.

Onyemata RO and Ibrahim EK, "Isolation andidentification of fungi andpathogenecity assessment of some spoilt fruits sold in Wuse market, Abuja, Nigeria," *Int. J. Curr. Res.*, 10(12): 76256–76259, 2018, [Online]. Available: doi: https://doi.org/10.24941/ijcr.33568.12.2018.

Martins ML, Martins HM and Bernardo F, "Aflatoxins in spices marketed in Portugal," *Food Addit. Contam.*, 18(4): 315–319, 2001, doi: 10.1080/02652030120041.

[23] Bello OB, Habib U, Olawuyi OJ, Opeyemi AS, Alafe AH and Owoade TA. "Microorganisms causing post-harvest tomato (Solanum lycopersicum L.) fruit decay in Nigeria." *J. Entomol. Zool. Stud.*, 4(1): 374–377, 2016.

El-Marzoky HA and ShabanWI, "Studies on some Garlic Diseases during Storage in Egypt," *J. Plant Prot.*, 2(2014): 25–30, 2014.

Khatoon A, Mohapatra A and Satapathy KB, "Studies on fungi associated with storage rot of onion (Allium cepa L.) and garlic (Allium sativum L.) bulbs in Odisha, India," *Int. Res. J. Biol. Sci.*, 6(1): 19–24, 2017.

Nahar N, Das S, Sufian A and Islam K, "Prevalence of Microbial Loads on Betel Leaf with Special emphasis on Multidrug Resistance Salmonella spp and its Public Health Implications," *Int. J. Environ. Agric. Biotechnol.*, 3(5): 1582–1589, 2018, [Online]. Available: http://dx.doi.org/10.22161/ijeab/3.5.3.

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