An Integrated Study of 'Black Head Moulds' Disease in Wheat (*Triticum aestivam*) Caused by *Alternaria triticina* with an Eco-friendly Management in Conventional Agro-climatic Approach

[1]Tanmay Ghosh, [2]Shamim Alam, *[3]Sandipan Chatterjee

[1]Department of Microbiology, Dinabandhu Andrews College, Baishnabghata, South 24 Parganas, Kolkata– 700084, West Bengal, India
Tanmay.tanmay.ghosh780@gmail.com
[2]Department of Botany, Suri Vidyasagar College, Suri-731101, Birbhum, West Bengal, India.
shamim3004@gmail.com
[3]Department of Botany, Suri Vidyasagar College, Suri, Birbhum – 731101, West Bengal, India.
chatterjee.sandipan1@gmail.com

ABSTRACT

The In-vitro laboratory experiment was conducted to valuate the efficacy of Indian Medicinal Plant like Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) by 'Agar Disc Diffusion' methodology. Beside of that a study was held parallel to exhibit the Concern of Eco friendly treatment as compared to Chemical fertiizer/pesticide, that going to be uncontrolled threat to us upon agro climatic environment. In this Integrated study we obtained antifungal activity resulted as 2.93±0.78 mm of 400mg/ml Ethyl acetate extract of Neem (*Azadirchata indica*) beside of that the other one determinated as 2.35±0.23mm of 400mg/ml Ethyl acetate extract of Kalmegh (*Andrographis paniculata*) followed by 2.10±0.45mm of Bel (*Aegle marmelos*) on the same concentration as compared to very high and effective rather than those Chemical Hazards.

Key Words: Black Head Moulds; Chemical fertilizers; *Alternaria triticina*; Agar Disc Diffusion; IC_{50} value; Antibiotics.

DOI: 10.48047/ecb/2023.12.9.148

INTRODUCTION

Black (sooty) head mold of wheat and barley is commonly in association with a diverse group of saprophytic or weakly parasitic fungi (Bockus et al. 2010).The typical symptom of black head mold in wheat and barley is the blackened appearances of mature or dead spikes under wet or humid weather conditions (Prescott et al. 1986). The presence of Stemphylium species with black head mold symptoms and grain discoloration of cereals have been reported in several publications (Zillinsky 1983; Prescott et al. 1986; Sisterna & Sarandon 2010; Hershman 2011; Zare 2013).Wheat (Triticum aestivum) stands as one of humanity's foundational crops, sustaining populations across the globe for centuries. Its significance in providing nourishment, sustenance, and economic stability cannot be overstated. However, the path to abundant wheat yields is laden with challenges, one of which is the insidious 'Black Head Moulds' disease caused by the fungus Alternaria triticina. This disease has far-reaching consequences, compelling agricultural communities to seek innovative and sustainable solutions to safeguard their harvests. Alternaria triticina, a fungal pathogen, orchestrates the 'Black Head Moulds' disease in wheat. Recognized by the characteristic darkening of wheat heads, this malady casts a shadow over grain development and quality, thereby undermining the productivity of wheat cultivation. The optimal conditions for Alternaria triticina's

proliferation are synonymous with humid environments, rendering regions susceptible to this disease particularly vulnerable. As modern agriculture grapples with the delicate balance between productivity and environmental stewardship, there has been a notable shift towards embracing eco-friendly management practices that coalesce with conventional agro-climatic realities. This paradigm shift embodies the ethos of sustainable agriculture, aiming to mitigate disease impact while minimizing the ecological footprint of traditional farming methods. The eco-friendly management of 'Black Head Moulds' disease in wheat within a conventional agro-climatic context transcends a single methodology and encompasses a holistic approach. At its core, this approach entails a harmonious integration of strategies that synergistically tackle the intricate web of disease dynamics while bolstering the overall agro-ecosystem resilience. The various fungi that cause black head mold primarily live on dead plant tissue and are typically only a problem when wheat dies prematurely. Also known as sooty head molds, black head molds appear dirty and come in a variety of colors (black, white, pink, or green) depending on the causal fungi. Growing on dead leaves, stems, and heads, these fungi are of most concern when they occur on heads. If kernels become infected by certain fungi and weather is favorable, they will develop a discoloration of the seed known as black point. The best time to scout is towards the end of the season during flowering and ripening. Some species of sooty molds produce as many as three different asexual forms (Hughes 1976). The mycelium of a sooty mold is composed of a weft of dark hyphae, which may be several millimeters thick. Conidiogenous cells and various types of conidia are formed within the mycelium. In some species, the weft of hyphae apparently is unorganized, whereas in other species the mycelium is highly organized and has a characteristic appearance (Parbery and Brown 1986). The conidia vary in size, shape, septation, and pigmentation. Species of the Metacapnodiaceae produce distinctive, tapering, moniliform hyphae with rigid smooth-walled cells. The colonies are dense and lustrous (Parbery and Brown 1986). Another sooty mold in the family Seuratiaceae produces dark, gelatinous, lobed thalli, with reproductive structures quite different from those of other sooty molds (Parbery and Brown 1986). The aim of the current study is to analyze the Eco-friendly treatment of Black Head Moulds disease in Wheat (*Triticum aestivam*) caused by *Alternaria triticina* and it's management through Ethyl Acetate extracts of different concentration of some Medicinal Plant like Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*). and this study also depicts the variation and helpfulness of using eco friendly treatment instead of Chemical fertilizers like Tricyclazole, Propiconazole and Mancozeb. In recent years, there has been growing interest in adopting eco-friendly management approaches to control plant diseases while minimizing the ecological impact of conventional agricultural practices. This approach aligns with sustainable agriculture principles and aims to reduce the reliance on synthetic chemical treatments. Eco-friendly management of 'Black Head Moulds' disease in wheat within a conventional agro-climatic context involves the integration of various strategies that collectively contribute to disease suppression. These strategies not only target the pathogen but also focus on enhancing the overall health of the agro-ecosystem.

MATERIALS AND METHODS

Sample Collection

The infected wheat seed sample was collected from farmers of Chinsurah, a agro facilated area in Hooghly District of State West Bengal and the same was Checked and identified by Prof. Tanmay Ghosh, Assistant Professor in Microbiology, Dinabandhu Andrews College, Garia, Kolkata, WB. IN.

Collection of Medicinal Plants

The Medicinal Plants Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) was collected from various location from Tarakeswar in Hooghly District of State West Bengal and the same was Checked and identified by Prof. Tanmay Ghosh, Assistant Professor in Microbiology, Dinabandhu Andrews College, Garia, Kolkata, WB. IN.

Collection of Chemical Fertilizer

The Chemical fertilizers like Tricyclazole, Propiconazole and Mancozeb was collected from Local Pesticide shop inTarakeswar, Hooghly, West Bengal and the same was Checked and identified by Prof. Tanmay Ghosh, Assistant Professor in Microbiology, Dinabandhu Andrews College, Garia, Kolkata, WB. IN.

Preparation of Plant extract

At first the plant structure (leaf) was washed with H_2O, dried in shade, grinded to fine powder and hold on in air tight containers at natural room temperature in dark till used. The fine-grained samples were subjected to extraction. 2g of air dried powder of Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) leaf extract was mixed with 5 ml Ethyl Acetate to obtain a final concentration of 400 mg/ml. Each solution was stored at 4°C after collecting in sterilized glass tubes until use.

Preparation of extract concentration

Four concentrations (50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml) were made of Ethyl Acetate extract. In each case 2gm of Extract was mixed with 5ml DMSO (Dimethyl sulfoxide) to organize four hundred mg/ml stock concentration. Then different 3 concentrations were created by adding further DMSO with the Stock in different tubing. Besife of that the concntration of chemical fertilizers was made by dissolving 0.5g and 1g sample in 10ml distilled water (dH_2O) respectively to obtain concentration of 0.05mg/ml and 0.1mg/ml.

Antifungal activity by 'Well plate diffusion method'

Muller Hinton agar was use to check antimicrobial activity by well diffusion method. Autoclaved medium was poured in to petriplates in the laminar air flow hood. On cooling the medium within petriplates the isolated *Alternaria triticina* from 24 hrs old culture were spread then wells were made on the petriplates with the help of stainless steel borer of diameter 6- 8 mm. Five plates were also made for each microorganism and one wall (Two part) made on each plate the entire surface at angle 180^o , Two plate was for four type of concentration (400 mg/ml, 200 mg/m; 100 mg/ml, 50 mg/ml) of each extracts Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*), Two plate is for same type of concentration (as the leaf extract) of each type of Chemical fertilizers like Tricyclazole, Propiconazole and Mancozeb and one for Control (by DMSO). Because we have to prove the extracts have large zone of inhibition of fungi against medicinal plants than those Chemical fertilizers by this parallel study. These plates were incubated for 48 hrs and the diameter of zone of inhibition was measured.

Statistical Analysis

Results obtained were analyzed statistically and values were expressed as Mean ± SD.

RESULTS AND DISCUSSIONS

Resulting antifungal activity

The Ethyl Acetate with grinned leaf of Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) were extracted to test the antifungal activity on the *Alternaria triticina* Fungi isolated from Black Head Moulds disease in Wheat (*Triticum aestivam*) with respect to standard antibiotic by the "agar well diffusion method" and the diameter zone of inhibition was measured in 'mm' parameter. Antifungal activity of extract with different concentrations on the *Alternaria triticina* is given as in Table 1 and Figure 1.

Table 1 : Diameter (mm) of inhibition of fungal growth for different medicinal plant with Ethyl Acetate extract on exact concentration (mg/ml).

Concentrations (mg/ml)	**Diameter (mm) of inhibition**		
	Kalmegh (*Andrographis paniculata*)	**Neem (*Azadirchata indica*)**	**Bel (*Aegle marmelos*)**
400	2.35±0.23	2.93±0.78	2.10±0.45
200	1.98±0.25	2.11±0.72	1.55±0.42
100	1.40±0.23	1.75±0.70	1.01±0.45
50	1.03±0.22	1.21±0.71	0.96±0.41
CONTROL (DMSO)	0.56±0.1	0.56±0.1	0.56±0.1

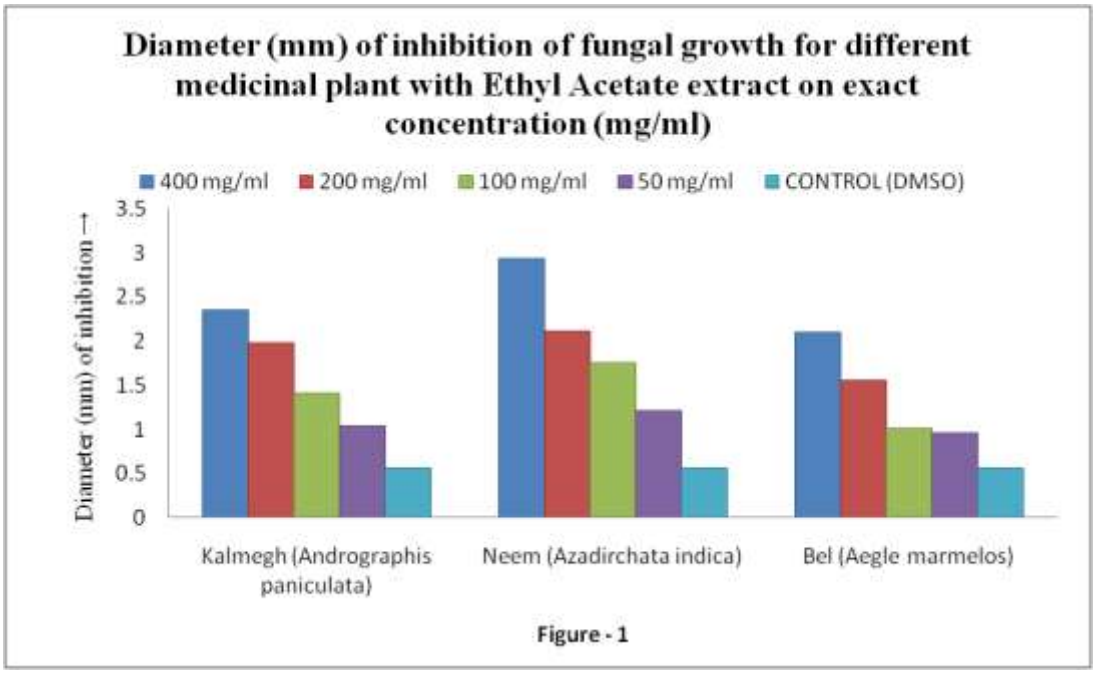


Figure - 1

With doing parallal study we have proved that The Chemical fertilizers (Tricyclazole, Propiconazole and Mancozeb) that cures naturally the Black Head Moulds disease in Wheat (*Triticum aestivam*) have less zone of inhibition as the Ethyl acetate extracts of Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata*

indica) and Bel (*Aegle marmelos*) have. The zone of inhibition of those Chemical fertilizers is given as Table 2.

Table 2 : Diameter (mm) of inhibition of fungal growth for Chemical fertilizers on exact concentration (mg/ml).

Concentrations (mg/ml)	**Diameter (mm) of inhibition**		
	Tricyclazole	**Propiconazole**	**Mancozeb**
0.05	1.34±0.54	1.56±0.15	1.02±0.33
0.1	2.01±0.55	2.11±0.16	1.99±0.34
CONTROL (DMSO)	0.56±0.1	0.56±0.1	0.56±0.1

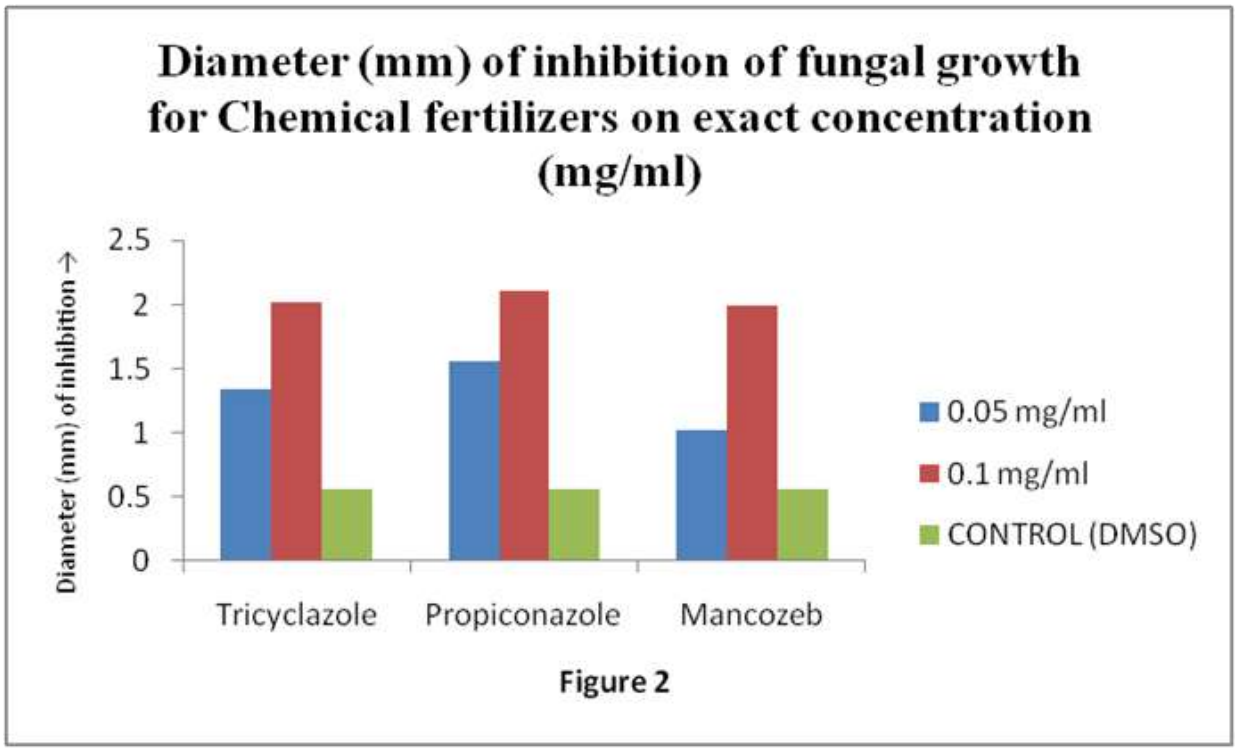


Figure 2

Here we can easily determinate that the Ethel Acetate extract of Neem (*Azadirchata indica*) is more effective than Kalmegh (*Andrographis paniculata*) followed by Bel (*Aegle marmelos*). on the basis of above mention analysis the most efficient effect of antifungal activity resulted as 2.93±0.78 mm of 400mg/ml Ethyl acetate extract of Neem (*Azadirchata indica*) beside of that the other one determinated as 2.35±0.23mm of 400mg/ml Ethyl acetate extract of Kalmegh (*Andrographis paniculata*) followed by 2.10±0.45mm of Bel (*Aegle marmelos*) on the same concentration and lower value evaluated as 0.96±0.41mm of Bel (*Aegle marmelos*) considered respectively by Control valued 0.56±0.1mm. On the other hand Here is some another comparing study, that proves the Ethyl acetate Extracts of Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) have much antifungal activity than the Chemical pesticides aganst Black Head Moulds disease in Wheat (*Triticum aestivam*)

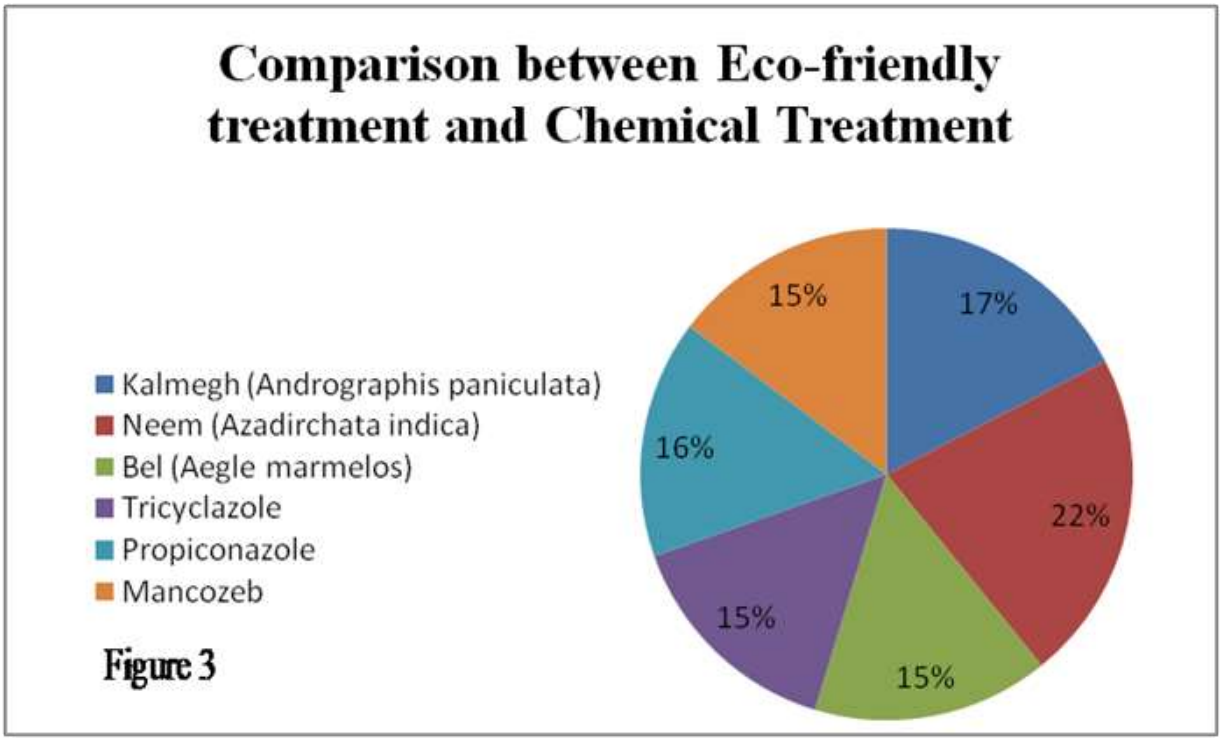
caused by *Alternaria triticina*. Graphical evaluation upon above discusion is given below as Figure 3, Determining the highest concentrations of each attempts.

IC_{50} Screening of above evaluation :

As we know IC_{50} represents the concentration at which a substance exerts half of its maximal inhibitory effect. This value is typically used to characterize the effectiveness of an antagonist in inhibiting a specific biological or biochemical process, the higher IC_{50} value depicting lower antifungal value respectively the determination is opposed to lower value.

Table 3 : IC_{50} value of each antifungal agents.

Highest concentration (mg/ml) used in inhibitory effect	**IC_{50} value**
Kalmegh (*Andrographis paniculata*)	3.05
Neem (*Azadirchata indica*)	1.23
Bel (*Aegle marmelos*)	5.66



Significantly we can conclude on the basis of Table 3 and Figure 4 that Neem (*Azadirchata indica*) is most efficient antifungal agent for *Alternaria triticina* compared to Kalmegh (*Andrographis paniculata*) following by Bel (*Aegle marmelos*).

CONCLUTIONS

Both barley that is still in the field and wheat that was not sprayed with fungicides are beginning to blacken in the field. The blackening of the heads is caused by several fungi that are referred to as “sooty molds”. Sooty mold fungi colonize wheat heads when wet, humid weather occurs during the latter stages of grain

development and crop maturation. Molding is frequently most severe when harvest is delayed. We know that organisms are gaining resistance day by day towards the multi drug or antibiotics, so that some natural product should be try to overcome these antibiotic resistant organisms. Moreover the plant extract of Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) have several antifungal efficacy against Black Head Moulds disease in Wheat (*Triticum aestivam*) caused by *Alternaria triticina* That's why we can say that the Ethyl Acetate extract of Kalmegh (*Andrographis paniculata*), Neem (*Azadirchata indica*) and Bel (*Aegle marmelos*) can be used for designing several drugs to treat complicated Black Head Moulds disease in Wheat (*Triticum aestivam*) in future.

REFERENCES

1. Bockus WW, Bowden RL, Hunger RM, Morrill WL, Murray TD, Smiley RW. 2010.Compendium of wheat diseases and pests, 3rd ed. APS Press, St. Paul, Minnesota, USA.
2. Zare L. 2013. The causal agent of barley black point disease in certified seed loads in Iran.International Journal of Agriculture and Crop Sciences 5: 332.
3. Zhong S, Steffenson BJ. 2001. Virulence and molecular diversity in Cochliobolus sativus. Phytopathology 91: 469–476.
4. Zillinsky FJ. 1983. Common diseases of small grain cereals. a guide to identification. CIMMYT, Mexico.
5. Hosen MI, Ahmed AU, Zaman J, Ghosh S, Hossain KM. 2009. Cultural and physiological variation between isolates of Stemphylium botryosum the causal of Stemphylium blight disease of lentil (Lens culinaris). World Journal of Agricultural Sciences 5: 94–98.
6. Sisterna M, Sarandón S. 2010. Wheat grain discoloration in Argentina: Current Status. The American Journal of Plant Science and Biotechnology 3: 54–64.
7. ISTA. Seed health testing methods and the germination test. In. International Rules for Seed Testing. Pub. by Intl. Seed Test. Assoc. Bassersdorf, Switzerland. Spring wheat. J. Phytopathol. 149: 185-188 (2005).
8. Warham, E.J., Effect of *Tilletia indica* Infectionon Viability, Germination and Vigour of Wheat Seed. Plant Disease, 74: 130-135 (1990).
9. Buchbauer G, Jirovetz L. Aromatherapy use of fragrances and essential oils as medicaments. Flav. Fragr. J., 1994; 9: 217 – 222.
10. Hussain A I, Anwar F, Sherazi S T H, Przybylski R. Chemical composition. Antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chemistry, 2008; 108: 986-995.
11. Mathur, P. Kapil, A. Das, B. and Dhawan, B. Prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. Indian J. Med. Res., 2002; 115: 153-157.
12. Sharma, S. Bhat, G.K. and Shenoy, S. Virulence factors and drug resistance in Escherichia coli isolated from extraintestinal infections. Indian J. Med. Microbiol., 2007; 25: 369-373.
13. Bahashwan, S.A. and El Shafey, H.M. Antimicrobial resistance patterns of Proteus isolates from clinical specimens. Eur. Sci. J., 2013; 9: 188-202.
14. Parekh, J. and Chanda, S. Antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. Turk. J. Biol., 2008; 32: 63-71.
15. Mahesh, B. and Satish, S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agri. Sci., 2008; 4: 839-843.

16. Ateb, D.A. and ErdoUrul, O.T. Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol., 2003; 27: 157-162.
17. Malthankar GV, White BK, Bhusan A, Daniels CK, Rodnick KJ, Lai JCK. Differential lowering by manganese treatment of activities of glycolytic and tricarboxylic acid (TCA) cycle enzymes investigated in neuroblastoma and astrocytoma cells is associated with manganese-induced cell death. Neurochem Res. 2004;29:707-717.
18. Althaf. In vitro anti- inflammatory activity of Vitex leucoxylon Linn. leaves by HRBC membrane stabilization, Int. J. of Pharm. & Life Sci. (IJPLS). 2013;4(1):2278-2281.
19. Mohamed Saleem TK. Anti-inflammatory activity of the leaf extacts of Gendarussa vulgaris Nees, Asian Pacific Journal of Tropical Biomedicine. 2011;147-149.
20. Darwish RM, Aburjai TA. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on Escherichia coli. BMC Complementary and Alternative Med. 2010;10(9):2-8.
21. Thenmozhi M, Vasuki K, Dhanalakshmi M, Devi K. Manjula. International Journal of Pharmacology and Biological Sciences. 2013;9-12.
22. Brindha P, Sasikala K, Purushoth K. Preliminary phytochemical studies in higher plants. Ethnobot. 1977;3:84-96.
23. Dosumu O, Nwosu VO, Nwogu ZCD. Antimicrobial studies and phytochemical screening of extracts of Hyphaene thebaica (Linn) Mart Fruits. International Journal of Tropical Medicine. 2006;1(4): 186-189.
24. Okigbo RN, Mbajiuka CS, Njoku CO, Antimicrobial potentials of (UDA) Xylopia aethopica and Occimum gratissimum L. on some pathogens of man. International J Molecular Medicine and Advance Science. 2005;1:392-397.
25. Sapirstein, H.D., David, P., Preston, K.R. and Dexter, J.E. (2007). Durum wheat bread making quality: Effects of gluten strength, protein composition, semolina particle size and fermentation time.Journal of Cereal Science, 45, 150–161.
26. Kaushik, R., Sharma, N., Swami, N., Sihag, M., Goyal, A., Anuj, P. and Chawla, P. (2013). Physico-chemical properties, extraction and characterization of gluten from different Indian wheat cultivars.Research and Reviews Journal of Crop and Crop Science, 2(1), 37-42.
27. Shrestha, K.K., Timla, R.D., Mahato, B.N., and Bim, H.B. (1998). Disease incidence and yield loss due to foliar blight of wheat in Nepal. In: Helminthosporium blights of wheat. Indian Journalof Mycology and Plant Pathology, 23(5):112-116.
28. Kapadiya, H.J. and Dhruj, I.U. (1999).Management of mung bean Cercospora leaf spot through fungicides. Indian Pytopathology, 52(1):96-97.
29. Vincent, J.M. (1927). Distortion of fungal hypha in presence of certain inhibitor nature, 159: 850.Singh, V.K., Singh, Gyanendra. And Bhattacharya, P.M. (2005). Incidence of Helminthosporium leaf blight of wheat and biochemical back-ground of disease resistance in the Eastern Gangetic Plains Journal of Wheat Research, 3(1): 26-28.
30. Sundar AR, Das ND, Krishnaveni D (1995). In- vitro Antagonism of Trichoderma spp. against two fungal pathogens of Castor. Indian Journal of plant protection, 23(2): 152-155.
31. Gharib, F.A. and Hegazi, A.Z. (2010). Salicylic acid ameliorates germination, seedling growth, phytohormone and enzymes activity in bean (Phaseolus vulgaris L.) under cold stress Journal of American Science, 6(10):675-683.

32. Rahmin MR. Rashid AQMB and Islam MA. 1999. Effect of seed treatment and foliar spray on the yield of wheat as affected by Bipolaris leaf blight. Bangladesh Journal of Plant Pathology 15: 17-9.
33. Shabana-Parveen, Kumar VR and Parveen S. 2000. Effect of extracts of some medicinal plants on the growth of Alternaria trinicina. Journal of Phytological Research 13(2): 195-6
34. Shrivastava A, Shrivastava M and Shrivastava A. 1998. Fungitoxic effect of some medicinal plants on some fruit pathogens. Philippine Journal of Science 127: 181-7.
35. Singh DP. Chand R. Dodan DS, Amerika Singh. Singh K. P. Tewari AN. Singh K M P, Satvinder Kaur, Singh R N, Singh AK. Singh S P. Singh V K. Brama R N, Kalappanawar 1 K. Solanki V A, Pathak R K, Pant S K. Das S Y and Chaudhary AK.2003. Evaluation of wheat genotypes for resistance to leaf blight caused by Bipolaris sorokiniona and Altemaria triticing Indian Phytopathology 56: 473-5
36. Singh DP. Sharma A K. Amerika Singh, Singh R V. Tewari AN Singh A K. Singh RN. Singh S P. Khanna B M. Dodan DS, Bagga PS and Kalappanavar 1 K.2002. Losses caused due to leaf blight in wheat in different agro-climatic zones of India. Plant Disease Research 17: 313-7.
37. Singh DP. Sharma AK, Tewari A N, Singh KP. Singh AK, Singh RN, Singh S P. Kalappanawar 1 K. Dodan DS and Singh V K.2004. Assessment of losses due to leaf blight in popular Varieties of wheat under different sowing conditions and agroclimatic zones in India. Indian Journal of Agricultural Sciences 74: 110-13.
38. Vincent J M. 1947. Distribution of fungal hyphae in presence of certain inhibitors. Nature 150: 850
39. Babana AH, Amadou HD, Kadia M, Traoré D (2012) Investigations on fungicidal sensitivity of Trichoderma spp. and Sclerotium rolfsii (collar rot pathogen) in crossandra. Internat J Appl Bio Pharm Techonol 2: 290-293.
40. Mamgain A, Roychowdhury R, Tah J (2013) Alternaria pathogenicity and its strategic controls. Res J Biol 1: 1-9.
41. Agale RC, Kadam JJ, Joshi MS, Borkar PG (2014) Symptomatology of purple blotch disease of onion and exploration of fungicides, phytoextract and bio-agents against causal fungus Alternaria porri. Hist 11: 63-69.
42. Aneja KR (2003) Experiment in Microbiology, Plant Pathology and Biotechnology. New Age International (P) Limited, Publishers. New Delhi, pp: 147-156.
43. Bhoyar PR, Chandankar VD, Bagde VL, Dipali B, Borkar Sonone JS (2014) Studies on seed borne m\coflora and eؤect of Bio-agents and fungicides on wheat seed health. Internat Quart J Life Sci Plant Pathol 9: 1285-1289.
43. Gharib FA, Hegazi AZ (2010) Salicylic acid ameliorates germination,seedling growth, phytohormone and enzymes activity in bean (Phaseolus vulgaris L.) under cold stress. J Am Sci 6: 675-683.
44. Halgekar SK, Ramachandra CG, Kalappanavar IK (2014) Evaluated the aؤect of dLؤerent antagonist viz. Trichoderma viride, Pseudomonas fluorescens and Bacilus substilis against dLؤerent seed brone fungi of rice. Internation J Agricul Sci 2: 503-504.