



EFFECT OF NEEM LEAF EXTRACT ON FUNGAL GROWTH

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Abstract Neem plant extract is one of the most important plant-derived extracts which is used as an herbal remedy and as a source of many therapeutic agents. The present paper deals with the effects of neem leaf extracts on fungal growth on bread sample. The efficacy of neem leaf extract was evaluated by treating the bread sample with raw extract, boiled extract and with a combination of both boiled and raw extract. Raw neem leaf extract was found to inhibit the growth of vegetative mycelium only, while the boiled extract was found to be fungitoxic, inhibiting spore formation and causing death of fungus. The result obtained from the combination of raw and boiled extract was not uniform and the effect was random. There was some new fungal growth at some regions in the bread piece treated with the combinations of raw and boiled extract and some regions showed dead fungus similar to the ones seen in the bread sample treated with boiled extracts. Therefore, the piece treated with boiled leaf extract was found to be more effective against fungal growth.

Key words: Neem leaf, bread, raw neem leaf extract, boiled neem leaf extract, efficacy analysis

1.Introduction

Neem extract has fungicidal effects against plant pathogenic fungi like *Alternaria solani*, *Fusarium oxysporum*, *Helminthosporium* species [3]. Experiment on effect of Neem (*Azadirachta indica* A. juss) leaf extract on the growth of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus fumigatus* isolated from foilar disease of Rice (*Oryza sativa*) [1, 5]. An experiment on inhibitory effect of aqueous and ethanolic extracts of Neem parts (leaves, stem, bark and seeds) on fungal rot disease of *Solanum tuberosum* were studied by [2,4,6]. They carried out the pathogenicity study and studied the effect of the plant extracts on mycelia growth of the test fungi using the food poisoning techniques. An experiment was conducted by [4,5] and the purpose of their study is to compare the efficacy of Neem extract and other

three antimicrobial agents incorporated into tissue conditioner in inhibiting the growth of *Candida albicans* and *Streptococcus mutans*. A study on effects of medicinal plant extracts and photosensitization on Aflatoxin producing *Aspergillus flavus* was conducted by Loise [5] This study was undertaken with an aim of exploring the effectiveness of medicinal plant extracts in the control of aflatoxin production. [3] conducted an experiment on inhibitory effect of aqueous and ethanolic extracts of Neem parts (leaves, stem, bark and seeds) on fungal rot disease of *Solanum tuberosum*. They carried out the pathogenicity study and studied the effect of the plant extracts on mycelia growth of the test fungi using the food poisoning techniques.

2. MATERIALS AND METHODS

2.1 MATERIALS

Brown bread

Fresh and healthy leaves of *Azadirachta indica*

Sealed plastic container / sealable plastic bag

Spray bottle

2.2 Collection of Plant Parts

Fresh and uninjured leaves of *Azadirachta indica* was collected from a healthy neem tree. The leaves were thoroughly washed under running tap water and then left to dry.

2.3 Food Sample

Fresh brown bread was taken and cut into 4-6 pieces then sprinkled with water so that the bread pieces absorbs moisture to stimulate the growth of the mold. The moistened pieces of bread were placed inside a sealed plastic container. The container was then stored in a dark, damp and warm area because mold thrives in warm environment with moisture in the air. Daily observation of the bread pieces were recorded along with photographs.

The 4-6 pieces of bread samples were made to study the effect of different types of leaf extracts on fungal growth, by selecting 4 bread samples with desirable fungal growth with visible colonies.

2.4 Plant Extract Preparation

From the fresh collected leaves, 50-60 healthy leaves were selected for the preparation of extract. Two types of extracts were prepared i.e., raw leaf extract and boiled leaf extract.

2.5 Raw Extraction

The raw extract was prepared by taking 30 healthy neem leaves and grinded by a grinder or pounded in mortar and pestle. 20ml of water was added to the crude extract and stirred well. The extract was then filtered using filter paper or a strainer.

2.6 Boiled Extraction

The boiled extract was prepared by taking 30 healthy neem leaves and boiled in 100ml of water. Neem leaves were boiled till the water turns green. The extract was then filtered using filter paper or a strainer and left to cool down.

2.7 Inhibition Test

After the proper growth of fungus on bread samples, which can be identified by the presence of white cottony thread like mycelia, black moldy spots, mini-mushroom like fruiting bodies, discoloration of the bread sample and a rotten pungent smell, it was then allowed to be treated with the prepared leaf extracts, to check the effect of each type of extract on the fungal growth.

In the first case, one of the bread pieces was kept as control sample, in which no extracts were added and the sample was allowed free fungal growth. In second case, another piece of bread sample was sprayed or sprinkled with the freshly prepared raw neem leaf extract. In the third case, one more piece of bread was sprayed or sprinkled with freshly prepared boiled neem leaf extract and in the fourth case; a mixture of the freshly prepared raw and boiled neem leaf extract was sprinkled. The container was again sealed and kept in its ideal place. Daily observations were recorded along with photographs.

3.RESULT

The bread samples placed in a condition favoring fungal growth, showed proper and desirable growth of microbes essential for the further experiment. Photograph of day 0 of the sample was taken when the bread samples were sprinkled with water and kept in dark, warm, humid place to initiate fungal growth. On day 1 not much of changes were seen only discoloration of the bread sample was observed. On day 3, initiation of fungal growth was seen. White mycelial growth along with black and yellow spotted growth can be observed. On day 4, growth of fungus has increased showing more white vegetative growth, black spots and some skin colored hairy growth. On day 5 fungal growths was extensive. Heavy growth of cottony white mycelium can be observed along with black spore formation.

The following figures show the day to day growth of the fungus:

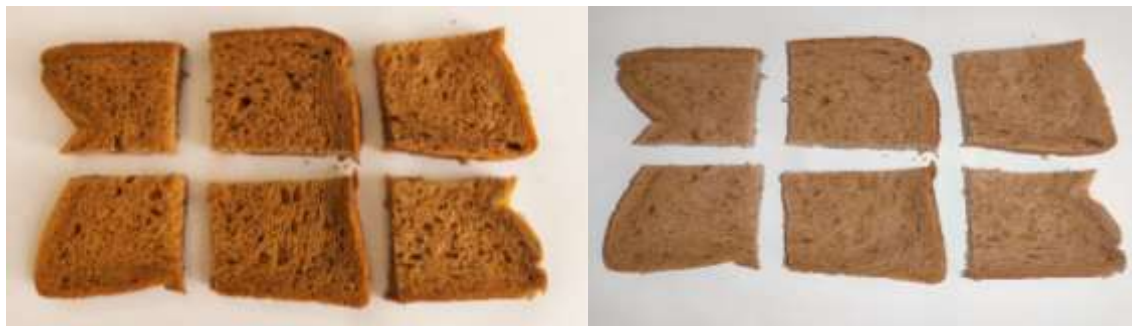


Fig 1:- Sample on day 0

Fig 2:- Sample on day 1



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Fig 3:- Sample on day 3

Fig 4:- Sample on day 4





Fig 5:- Sample on day 5

After the desirable fungal growth on the bread samples, the bread samples were taken for treatment with the extracts of neem leaf. Pictures of bread samples just after sprinkling the extracts were shown shown below in table 1:

TABLE 1

Fig 6: Sample 1	Fig 7: Sample 2
Control sample with no extract	Sample just after sprinkling raw extract

Fig 8: Sample 3	Fig 9: Sample 4
	
<p>Sample just after sprinkling boiled leaf extract</p>	<p>Sample just after sprinkling the mixture of raw and boiled leaf extract</p>



The bread samples after their treatment with the leaf extracts were kept at their usual places and were under observation. Samples were observed for three days after treatment and their effects were studied.



ControlSamples

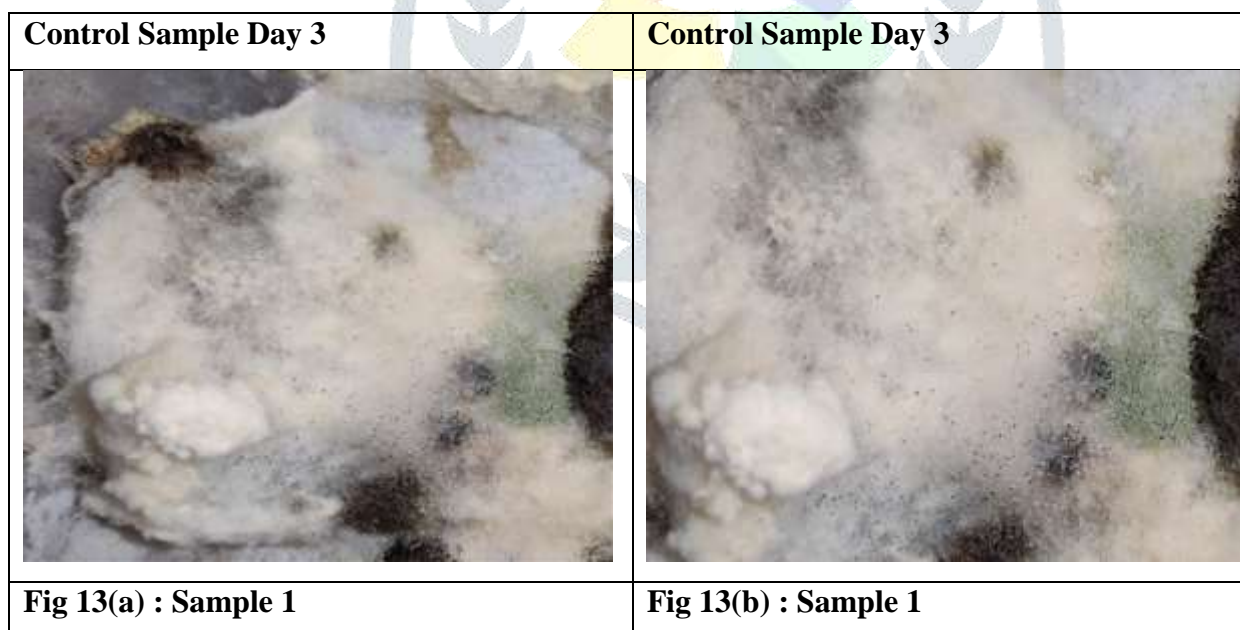
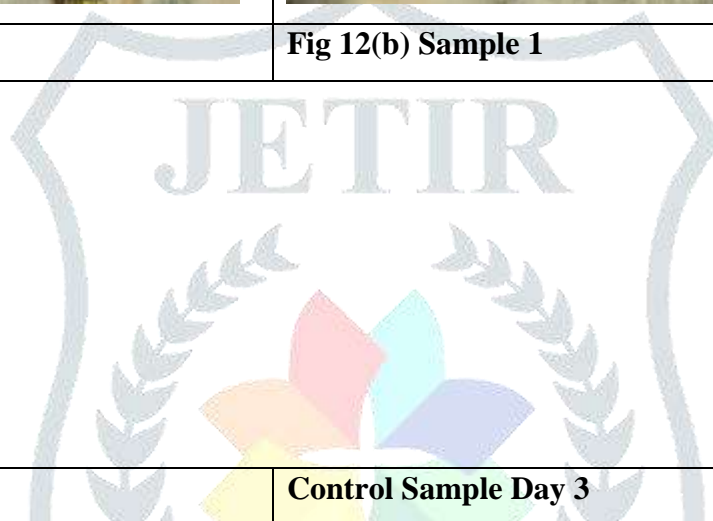
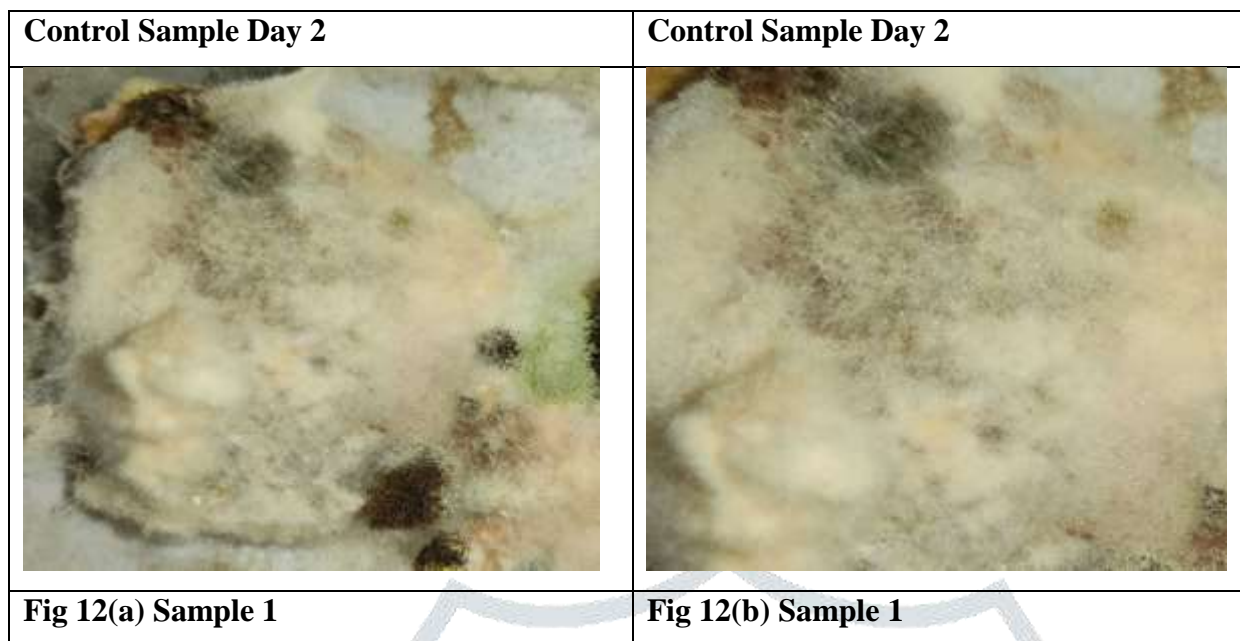
Control samples of all the three days under observation were studied and photographs were taken. It shows extensive growth of vegetative mycelium which is represented by much colonised cottony white growth. Sporulation has started.

The pictures of control samples from Day 0 to Day 3 are shown in table 2

TABLE 2

Control Sample Day 0	Control Sample Day 0
	
Fig 10(a) : Sample 1	Fig 10(b) : Sample 1

Control Sample Day 1	Control Sample Day 1
	
Fig 11(a) : Sample 1	Fig 11(b) : Sample 1







RawExtract

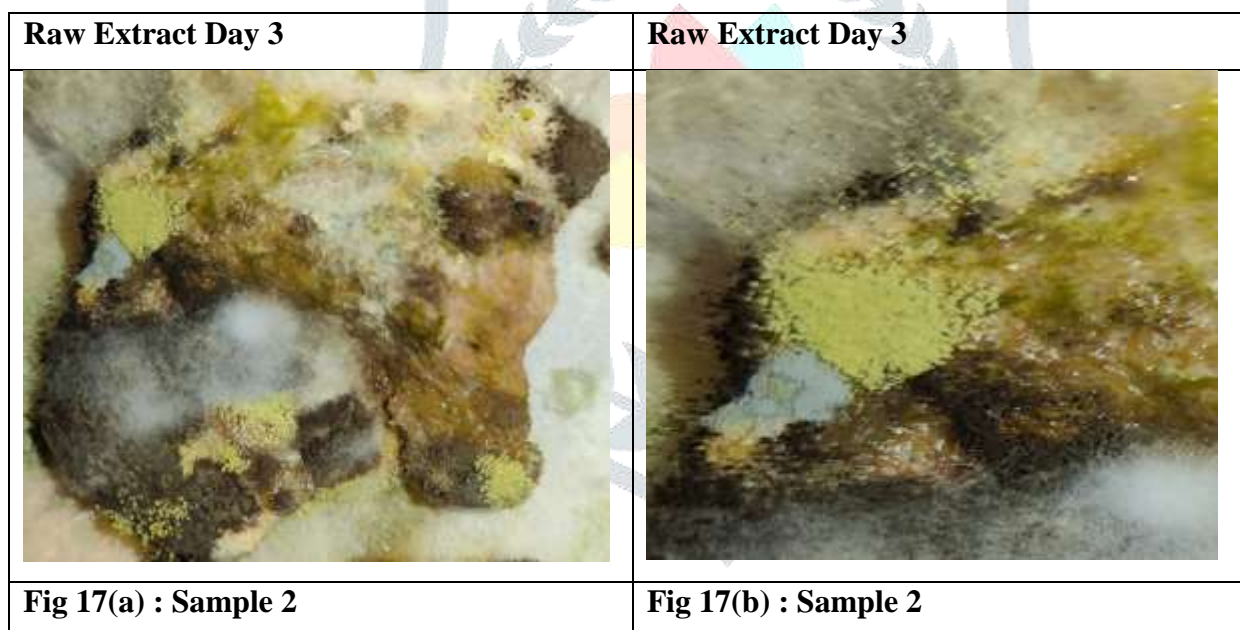
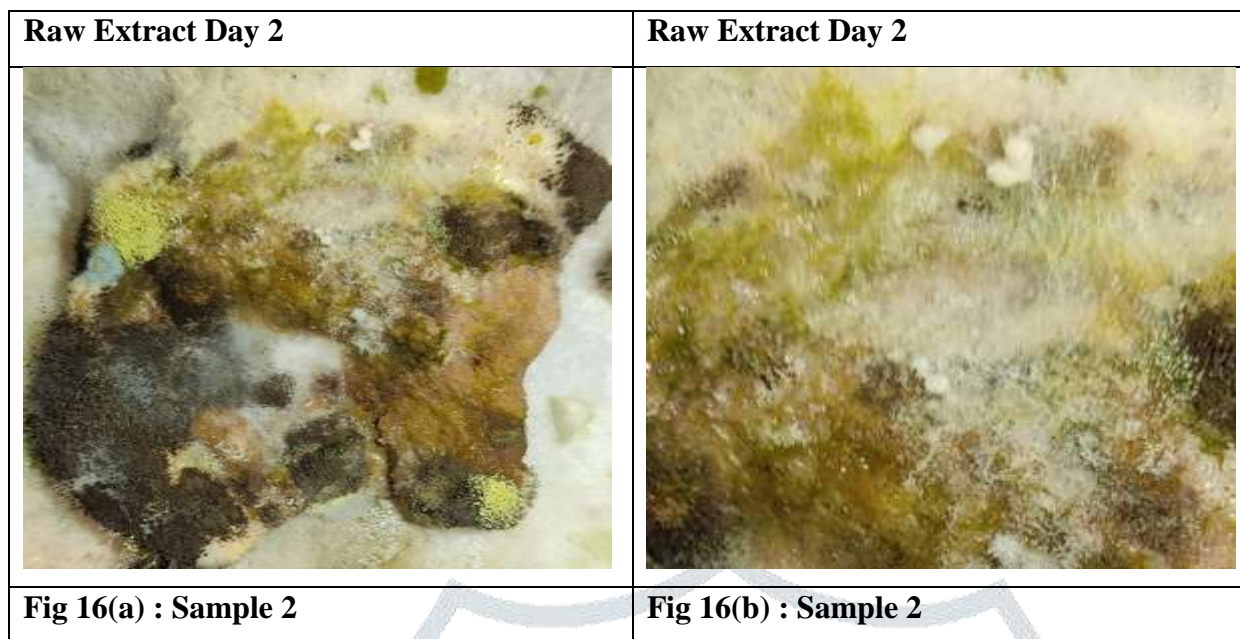
Sample treated with raw extract of all the three days under observation were studied and photographs were taken. The bread sample of Day 0 treated with raw neem leaf extract appears wet with and green because of the freshly prepared raw extract. On day 1, bread sample showed less mycelial growth, there was reduction in vegetative growth of the fungus. After 2nd day the growth of vegetative mycelium is further inhibited but

at some places more of spores were also seen. On day 3, new fungal species of green and grey color were observed.

TABLE 3

Raw Extract Day 0	Raw Extract Day 0
	
Fig 14(a) : Sample 2	Fig 14(b) : Sample 2

Raw Extract Day 1	Raw Extract Day 1
	
Fig 15(a) : Sample 2	Fig 15(b) : Sample 2





Boiled Extract



Samples treated with boiled neem leaf extract of all three days under observation were studied and photographs were taken. On day 0, the sample treated with extract appears wet with extract and the boiled extract seems colourless. On day 1 the piece shows the inhibition of spores. The black color spots represents the sporulation stage of fungus and dead fungus. The spores are peeling off and there is inhibition in the growth of fungus. On day 2, over the peeled off regions of spores small white velvet like colonies were formed. On day 3, inhibition of spore germination and death of fungus was observed. Over the peeled off



and dead spores the white growths have increased a little and some new fungal species with pigments were observed.



The pictures of samples treated with boiled neem leaf extract from Day 0 to Day 3 are shown in table 4 :

TABLE 4

Boiled Extract Day 0	Boiled Extract Day 0
	
<p>Fig 18(a) : Sample 3</p>	<p>Fig 18(b) : Sample 3</p>

Boiled Extract Day 1	Boiled Extract Day 1
	
<p>Fig 19(a) : Sample 3</p>	<p>Fig 19(b) : Sample 3</p>

Boiled Extract Day 2	Boiled Extract Day 2
	
Fig 20(a) : Sample 3	Fig 20(b) : Sample 3

Boiled Extract Day 3	Boiled Extract Day 3
	
Fig 21(a) : Sample 3	Fig 21(b) : Sample 3


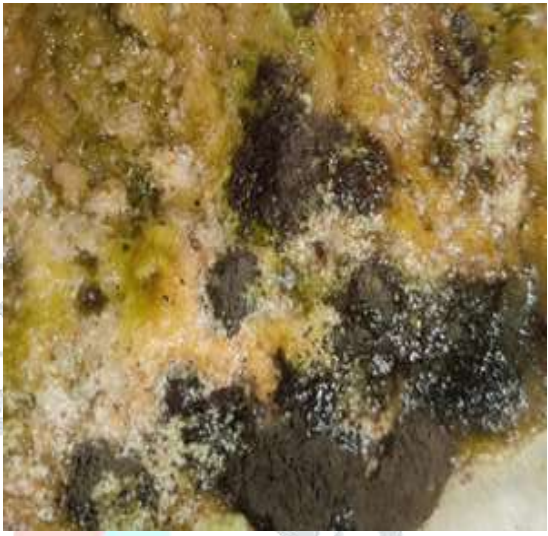
Combination Of raw and boiled Extract



Sample treated with combination of raw and boiled neem leaf extract of all three days under observations were studied and photographs were taken. On day 0, the bread sample appear wet and a slightly greenish because of the extract. On day 1 extract shows both inhibition of mycelial growth at some regions and death of fungus at other regions. On day 2, new growth of fungi white and grey color were observed in some regions. And on day 3, there was no change on the bread sample. The growth of fungus has not increased nor

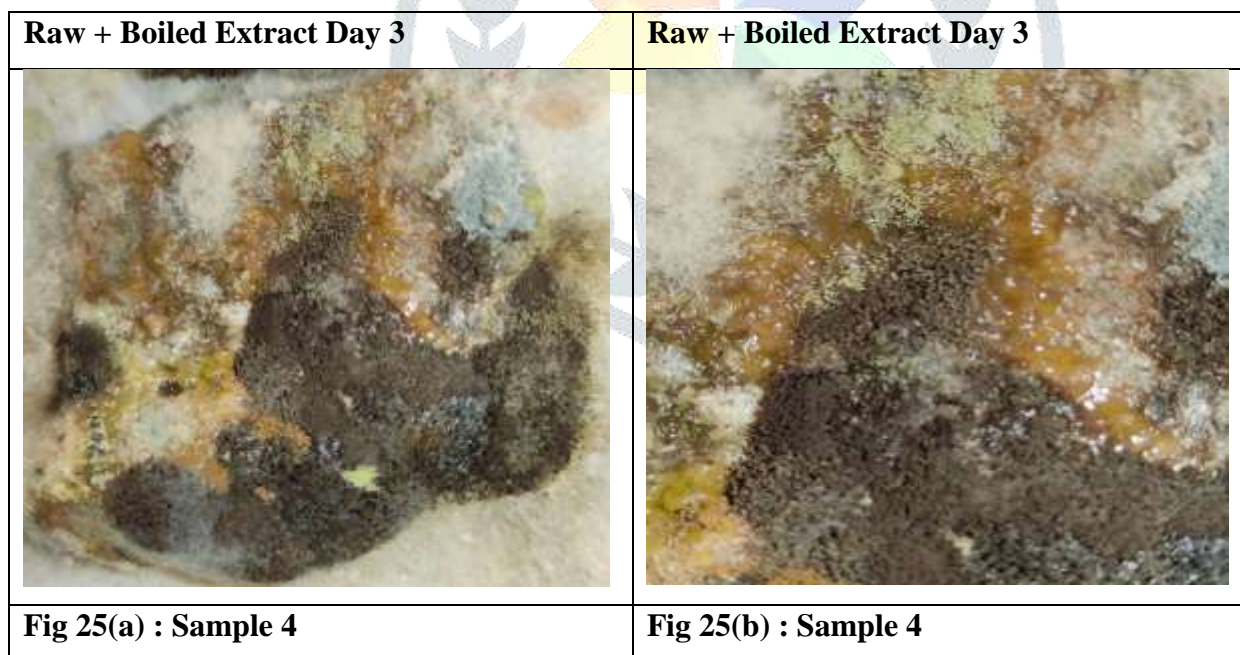
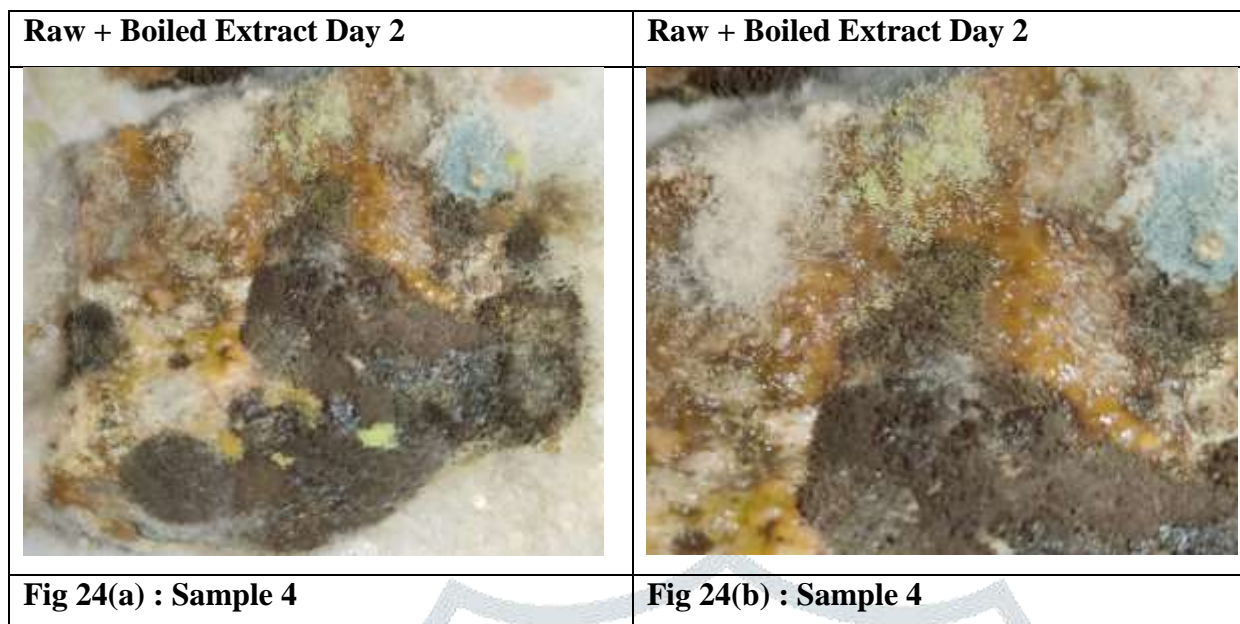
decreased than day 2. This shows the random effect of the combination extract of neem leaf, maybe due to uneven sprinkling of the extract or uneven mixing of raw and boiled extract.

The pictures of samples treated with combination of raw and boiled neem leaf extract from Day 0 to Day 3 are shown in table 5 :

TABLE 5

Raw + Boiled Extract Day 0	Raw + Boiled Extract Day 0
	
Fig 22(a) : Sample 4	Fig 22(b) : Sample 4

Raw + Boiled Extract Day 1	Raw + Boiled Extract Day 1
	
Fig 23(a) : Sample 4	Fig 23(b) : Sample 4



4. DISCUSSION

This study was conducted to investigate the efficacy of neem leaf extracts on fungal growth and to compare the efficacy of different types of neem leaf extract on the fungal growth. This experiment also showed the inhibition of fungal growth on bread pieces when treated with different leaf extracts of *Azadirachta indica*.

The inhibition rate of fungal growth by treating with raw extract, boiled extract and with a combination of both boiled and raw extract is different according to their concentration. Aqueous extract of various parts of neem such as neem oil and its chief principles have antifungal activities and have been reported by earlier investigators [1]. From the control bread sample it was observed that the day to day growth of vegetative fungal mycelium is extensive. While the treatment of bread sample with crude extract obtained from leaves inhibited both spore germination and vegetative mycelial growth of *Rhizopus*, *Penicillium*, *Mucor* and *Aspergillus* species. Aflatoxins are secondary metabolite produced by toxigenic strains of some *Aspergillus* species on food. *Azadirachta indica* is a known inhibitor of aflatoxin production [8]. Raw neem leaf extract was found to inhibit the growth of vegetative mycelium only, while the boiled extract was found to be fungitoxic, inhibiting spore formation and causing death of fungus. The result obtained from the combination of raw and boiled extract was not uniform and the effect was random. There was some new fungal growth at some regions in the bread piece treated with the combination of raw and boiled extract and some regions showed dead fungus similar to the ones seen in the bread sample treated with boiled extracts. Therefore, the piece treated with boiled leaf extract was found to be more effective against fungal growth.

This study can be found to many limitations. Though it was concluded that boiled extract obtained from leaves of *Azadirachta indica* was more effective than the raw and mixture of raw and boiled extract but failed to show the complete inhibition of fungal growth, however it showed that neem extracts affected the growth rate and sporulation of fungi, even though their mode of action is not fully understood. Additional research is needed to determine the potential usefulness of neem extracts and products in fungal control programs.

5. REFERENCES

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