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Antifungal activities of some medicinal plant extracts against *Colletotrichum gloeosporioides* (die-back) and *Pestalotia theae* (grey blight) of tea

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ABSTRACT: The research work was carried out to evaluate the antifungal activities of leaf extracts of four medicinal plants *viz. Azadirachta indica* (Neem), *Polygonam barbatum* (Bishkatali), *Adhatoda vasica* (Basak) and *Terminalia arjuna* (Arjun) against two tea fungi *Colletotrichum gloeosporioides* (Die-back) and *Pestalotia theae* (Grey blight). Leaf extracts of these plants were prepared by using distilled water, ethanol absolutes (95%) and ethanol (70%) as solvents. After that, three concentrations (1%, 1.5% and 2%) of leaf extracts of these four plants were prepared. The antifungal activities of crude extracts were investigated by the poison food technique against these two experimental fungi. The results showed that extracts of all the four plants had direct effect on both fungi. Among all the concentrations of leaf extracts of the four experimental plants diluted in distilled water against *Colletotrichum gloeosporioides*, all concentrations of *Adhatoda vasica* (Basak) exhibited the highest growth inhibition (100%) and *Polygonam barbatum* (Bishkatali) at 1% concentration showed the lowest growth inhibition (38.88%). And against *Pestalotia theae*, *Adhatoda vasica* (Basak) at 2% concentration showed the highest effect (86.66%) and *Polygonam barbatum* (Bishkatali) at 1% concentration exhibited the lowest effect (05.55%). As all the concentrations of leaf extracts of respective plants diluted in ethanol absolutes (95%), all leaf extracts at all concentrations showed the same growth inhibition (100%) against *Colletotrichum gloeosporioides*, and against *Pestalotia theae*, *Azadirachta indica* (Neem) 2%, *Polygonam barbatum* (Bishkatali) 2%, *Adhatoda vasica* (Basak) at all concentrations, and *Terminalia arjuna* (Arjun) 2% showed the highest growth inhibition (100%) and *Polygonam barbatum* (Bishkatali) 1% exhibited the lowest effect (97.11%). Among all the concentrations of leaf extracts of the said plants diluted in ethanol 70% against *Colletotrichum gloeosporioides*, all concentrations of *Adhatoda vasica* (Basak) showed the highest growth inhibition (100%) and *Terminalia arjuna* (Arjun) 1% exhibited the lowest effect (91.11%). And against *Pestalotia theae*, *Adhatoda vasica* (Basak) at 1.5% and 2% concentrations showed the highest antifungal effect (100%) and *Terminalia arjuna* (Arjun) 1% showed the lowest effect (90%). In this study it was investigated that among all concentrations of respective plants diluted in all solvents, *Adhatoda vasica* (Basak) exhibited the highest effect on both fungi and *Polygonam barbatum* (Bishkatali) showed the lowest growth inhibition. Among the solvents, ethanol extracts showed more growth inhibition of both fungi than other leaf extracts. From this research work this is evident that all the plant extracts are potential antifungal agents against experimental both tea fungi, and the effectiveness of most of the plant extracts increases proportionally with the increase in doses and decreases with time.

Keywords: Tea (*Camellia sinensis*); Leaf extracts; Antifungal activity; *Colletotrichum gloeosporioides*; *Pestalotia theae*

Introduction

Tea (*Camellia sinensis*) is the single most important cash crop that has been contributing a lion's share to the Bangladesh economy. Although it plays significant role in the economy of the country, but the crop suffers from many production constraints.

Tea diseases are one of the most constraints for the production of tea. Different pathogens like bacteria, algae, viruses and fungi cause tea diseases. Fungi like *Colletotrichum gloeosporioides* and *Pestalotia theae* are responsible for tea diseases like Die-back and Grey blight respectively. Die-back of tea is one of the major diseases in Bangladesh tea both in mature and nursery plants. During the late 1960s, it is recorded on the India clone TV9 and reported to be widespread in tea, in permanent and temporary shade trees, green crops as well as in rubber plantations in Bangladesh.[1] Die-back of shoots of tea, coffee and cocoa is reported in India, Malawi and Sri Lanka. The causal organism of the disease in tea is *Colletotrichum gloeosporioides* (Penz.), Sacc.[1,2] Besides these, *C. camelliae* Mass is reported to cause Brown blight in tea in India and Africa.[3,4]

The fungi Colletotrichum was established in 1831 by Corda (1831), for fungi characterized by hyaline, curved, fusion conidia and setose acervuli. Diseases caused by Colletotrichum species occur on a wide range of plant species and have been recorded worldwide.[5] Colletotrichum species are by far the most important pathogens

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which cause this type of infection. Die-back disease of tea was first noticed in the year 1984 in Nucleus Clone Plot (NCP) of Bangladesh Tea Research Institution (BTRI) on the clone BT4. More than 40% of tea bushes were found to be attacked by the disease. In the same year the disease was reported in Tipracherra tea estate where 30% of the seed nursery beds were affected. About 55–60% of the plants died due to this disease. Though the disease was first noticed in the NCP, later the same disease was also found in the nursery as well as mature plantation. Visibility of the disease infection and the extent of damage apparently escaped the notice in mature plantation because of continuous plucking during the harvesting period. Since then, the disease is appearing every year with a variation in its intensity depending on climatic factors.[6] Besides tea, the pathogen has been isolated and reported on tea ancillary plants like – *Albizzia anagroides*, *A. moluccana*, *Mimosa pudica*, *Indigofera teysmnii*, *Crotolaria anagyroides*, *Tephrosia candida*, Coffee, Bay leaf, Rubber and different citrus species including orange.[7] Another major foliar disease of tea, Grey blight, is caused by *Pestalotia theae*. Crop losses due to Grey blight are about 17% in south India.[8]

Medicinal plants act as a rich source of anti-microbial agents. They were used as medicinal products in many countries due to the potent source of the powerful drugs. The raw drugs obtained from various parts have different medicinal properties.[9] The medicinal plants were still used by the developing countries as the traditional folk medicine due to the biologically active compounds hidden in it, and this concept that drives the scientists working towards them in a dedicated manner.[10] Antifungal agents are widely distributed among higher plants,[11] but only a few have been evaluated for their activity against human, animal and plant pathogenic fungi. Fungi are of the most neglected pathogens, as demonstrated by the fact that the amphotericin B, a polyene antibiotic discovered as long ago as 1956, is still used as a gold standard for antifungal therapy.[12] The fungal growth may cause decrease in germinability, discolouration of grain, loss in weight, biochemical changes and production of toxins.[13] Climatic conditions are most conducive for mould invasion, elaboration of mycotoxins. Unseasonal rains and floods enhance the moisture content of the grain, making them more vulnerable for fungal attack.[14] The present study was undertaken to invastigate the suitable plant extracts and their concentrations for controlling of *Pestalotia theae* and *Colletotrichum gloeosporioides* and to determine the antifungal activity of crude extracts of *Azadirachta indica*, *Polygonam barbatum*, *Adhatoda vasica* and *Terminalia Arjuna* against *Colletotrichum gloeosporioides* and *Pestalotia theae* of tea.

Materials and Methods

Study Area

The research work was conducted under Research Project of "Antifungal activities of some medicinal plants extracts against *Colletotrichum gloeosporioides* and *Pestalotia theae* of tea" during the period of August–January 2011–2012 at the Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet, and at Plant Pathology Laboratory, Bangladesh Tea Research Institution (BTRI), Srimangal.

Collection of Plant Material

The fresh leaves of four medicinal plants *viz.* neem (*Azadirachta indica)*, bishkatali (*Polygonam barbatum)*, basak (*Adhatoda vasica)* and arjun (*Terminalia arjuna*) free from disease were collected from the different locations of Shahjalal University of Science and Technology campus and its surrounding. The leaves were washed thoroughly 2–3 times with running water and once with sterile distilled water. The washed plant leaves were air dried at room temperature for 48 hr, and then they were dried by dryer at 60°C for 6–8 hr to get constant weight. After that the dried leaves were powered with the help of a blender machine. The ground samples were passed through a 25-mesh diameter sieve to obtain fine and uniform dust. The dust was preserved in airtight condition in polythene bags till their use in extract preparation.

Preparation of Leaf Extracts

Plant leaf extracts were prepared by the method of Amadioha *et al.*[15] with some modification. Ten gram of dust of each plant leaves was taken in a 250-ml conical flux and mixed separately with 100 ml of different solvents like distilled water, ethanol absolutes (95%) and ethanol 70%. The mixture was stirred for 30 min by a magnetic stirrer (at 6,000 rpm) and left to stand for next 48 hr. The mixture was then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtrated materials were taken in 100 ml beaker and condensed to 10 ml by evaporation of solvent in a water bath maintained at 60°C, 70°C and 80°C for ethanol absolutes (95%), 70% ethanol and sterilized distilled water, respectively. After the evaporation of solvent, the condensed extracts were preserved in tightly corked labeled bottles, *e.g.* 50-ml

volumetric flux and stored them in a refrigerator until their use for antifungal activities. Different concentrations of plant extracts (1%, 1.5% and 2%) were prepared by dissolving the stock solutions in the respective solvent prior to study of antifungal activities.

Screening of Anti-fungal Activity

The antifungal activity was determined by the poison food technique with some modification.[16]

Collection of Fungul Sample

The fungi *Colletotrichum gloeosporioides* and *Pestalotia theae* were isolated from Die Back affected tea plants and Grey Blight affected tea plants at BTRI Farm, Bilashcherra Experimental Farm and Baraoora Tea Estates.

Preparation of Media

For preparing 1 L media, 1 L Distilled Water, Potato 200 g, Dextrose 20 g and Agar 20 g were used. The media was made treated by adding different concentrations (1%, 1.5% and 2%) of Neem, Bishkatali, Basak, Arjun leaf extracts with distilled water, ethanol absolutes (95%) and 70% ethanol. Then, 5 ml of lactic acid was added in the mixture.

Sterilization of Media and Other Glass Wares

Prepared media and glass wares were sterilized by autoclave for 15 min at 1 bar pressure.

Preparation of Inoculums

In the laboratory for each disease the infected portion of tea plants were cut along with slightly healthy parts. These were surface sterilized with 25% ethanol. After three times washing with distilled water, these inoculums were put in the centre of media and allowed at room temperature in Laminer air flow chamber for growth.

Inhibition Test

The PDA was poisoned with different concentrations of earlier-prepared extracts. Then, a bloc of fresh cultured fungus was transferred into poisoned media and allowed to grow. The radial growth of fungus for each treatment was determined by measuring the diameter of the colony every 24 hr of calculation on poisoned plate. This practice was performed three times for the same. The percentage radial growth inhibition was calculated as follows:

$$h = (C - E)/E \times 100$$

where h = percentage of growth inhibition of fungus; C = growth of fungus in control; E = growth of fungus in extract.

Table 1: Effect of leaf extracts of Neem (*Azadirachta indica*), Bishkatali (*Polygonam barbatum*), Basak (*Adhatoda vasica*) and Arjun (*Terminalia arjuna*) diluted in distilled water at different concentrations on the growth of *Colletotrichum gloeosporioides* and *Pestalotia theae* after 120 hr of plating

Treatments	Concentrations	Name of fungi			
		Colletotrichum gloeosporioides		*Pestalotia theae*	
		Mycelial growth (cm) Average of three replications	% Growth inhibition	Mycelial growth (cm) Average of three replications	% Growth inhibition
Azadirachta indica (Neem)	1.0%	5.00 d	44.44	8.00 d	11.11
	1.5%	4.86 e	46.00	7.30 f	18.88
	2.0%	4.50 g	50.00	3.50 k	61.11
Polygonam barbatum (Bishkatali)	1.0%	5.50 b	38.88	8.50 b	05.55
	1.5%	5.13 c	43.00	8.10 c	10.00
	2.0%	4.50 g	50.00	7.60 e	15.55
Adhatoda vasica (Basak)	1.0%	0.00 i	100.00	6.00 h	33.33
	1.5%	0.00 i	100.00	3.00 l	66.66
	2.0%	0.00 i	100.00	1.20 m	86.66
Terminalia arjuna (Arjun)	1.0%	5.00 d	44.44	7.00 g	22.22
	1.5%	4.63 f	48.55	5.80 i	35.55
	2.0%	4.06 h	54.88	4.10 j	54.44
Control	–	9.00 a	00.00	9.00 a	00.00

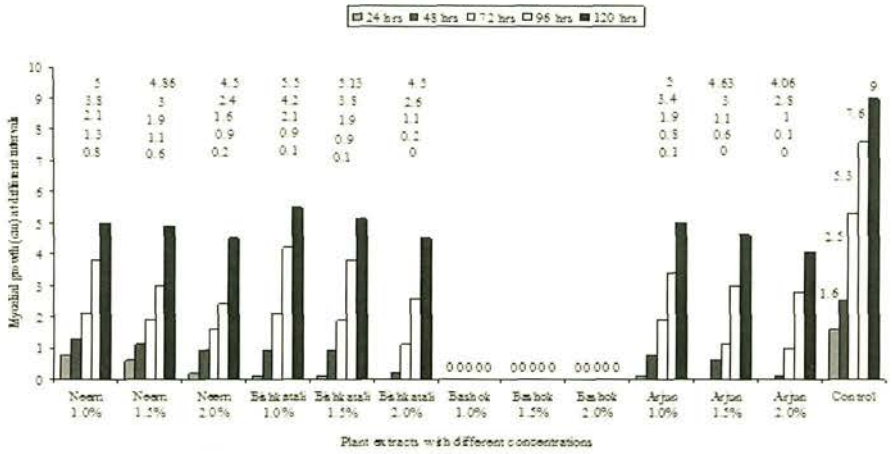

Figure 1. Mycelial growth of *Colletotrichum gloeosporioides* against some plant extracts diluted in distilled water with different concentrations at different intervals.

Results and Discussion

In the present study, four medicinal plants such as *Azadirachta indica* (Neem), *Polygonam barbatum* (Bishkatali), *Adhatoda vasica* (Basak) and *Terminalia arjuna* (Arjun) were identified that are effective against the two target fungi *Colletotrichum gloeosporioides* and *Pestalotia theae*. Leaf extracts of these plants were serially diluted in distilled water, ethanol absolutes (95%) and ethanol 70% (70 ml ethanol + 30 ml distilled water) and the percentage of growth inhibition values were determined (Tables 1–3). As shown, all these four plants have the potential antifungal activity to control the growth of both tea fungi.

Effect of Different Plant Extracts Diluted in Distilled Water on the Growth of *Colletotrichum gloeosporioides* (Die-back) and *Pestalotia theae* (Grey blight)

Table 1 showed that among all the concentrations of leaf extracts of *Azadirachta indica* (Neem), *Polygonam barbatum* (Bishkatali), *Adhatoda vasica* (Basak) and *Terminalia arjuna* (Arjun) the mycelial growth of *Colletotrichum gloeosporioides* (5.00 cm) statistically same for 1% concentrations of *Azadirachta indica* (Neem) and *Terminalia arjuna* (Arjun) leaf extracts. On the other hand, *Azadirachta indica* (Neem) and *Polygonam barbatum* (Bishkatali) for 2% concentration the mycelial growth was same (4.50 cm). The results also showed that for all the concentrations of *Adhatoda vasica* (Basak) there was no mycelial growth of *Colletotrichum gloeosporioides*. Whereas the pool variance analysed results showed also statistically different response on the mycelial growth of *Pestalotia theae*.

In this experiment, leaf extracts of *Adhatoda vasica* (Basak) for all the concentrations showed the highest growth inhibition rather than control of *Colletotrichum gloeosporioides* (100%) and against *Pestalotia theae*

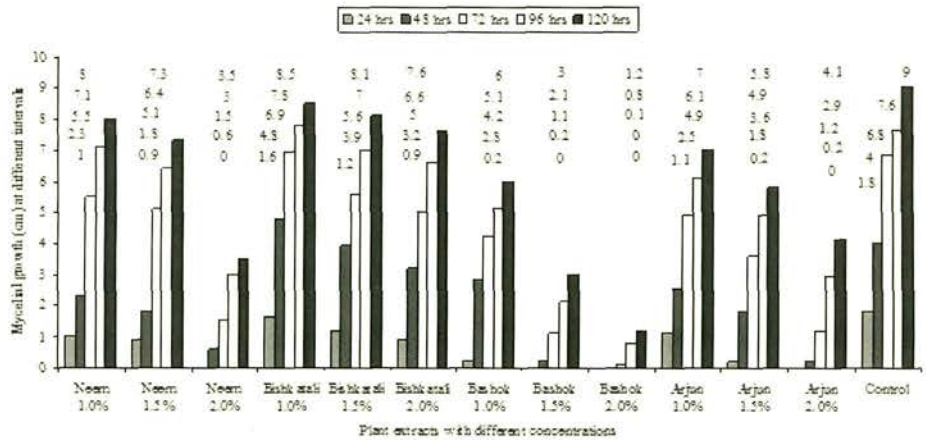

Figure 2. Mycelial growth of *Pestalotia theae* against some plant extracts diluted in distilled water with different concentrations at different intervals.

Table 2: Effect of leaf extracts of Neem (*Azadirachta indica*), Bishkatali (*Polygonum barbatum*), Basak (*Adhatoda vasica*) and Arjun (*Terminalia arjuna*) diluted in ethanol absolutes (95%) at different concentrations on the growth of *Colletotrichum gloeosporioides* and *Pestalotia theae* after 120 hr of plating

Treatments	Concentrations	Name of fungi			
		Colletotrichum gloeosporioides		*Pestalotia theae*	
		Mycelial growth (cm) Average of three replications	% Growth inhibition	Mycelial growth (cm) Average of three replications	% Growth inhibition
Azadirachta indica (Neem)	1.0%	0.00 b	100	0.20 c	97.78
	1.5%	0.00 b	100	0.06 d	99.33
	2.0%	0.00 b	100	0.00 e	100.00
Polygonam barbatum (Bishkatali)	1.0%	0.00 b	100	0.26 b	97.11
	1.5%	0.00 b	100	0.10 d	98.88
	2.0%	0.00 b	100	0.00 e	100.00
Adhatoda vasica (Basak)	1.0%	0.00 b	100	0.00 e	100.00
	1.5%	0.00 b	100	0.00 e	100.00
	2.0%	0.00 b	100	0.00 e	100.00
Terminalia arjuna (Arjun)	1.0%	0.00 b	100	0.06 d	99.33
	1.5%	0.00 b	100	0.00 e	100.00
	2.0%	0.00 b	100	0.00 e	100.00
Control	–	9.00 a	00	9.00 a	00

the highest growth inhibition was found 86.66% for 2% concentration of *Adhatoda vasica* (Basak). Accordingly the lowest growth inhibition of both fungi *viz. Colletotrichum gloeosporioides* (38.88%) and *Pestalotia theae* (05.55%) were observed for 1% concentration of *Polygonam barbatum* (Bishkatali).

Figures 1 and 2 showed a gradual increasing trend in mycelial growth of *Colletotrichum gloeosporioides* and *Pestalotia theae* for all the concentrations of said plant extracts diluted in distilled water with increasing time.

Effect of Different Plant Extracts Diluted in Ethanol Absolutes (95%) on the Growth of *Colletotrichum gloeosporioides* (Die-back) and *Pestalotia theae* (Grey blight)

Table 2 showed that all the concentrations (1%, 1.5% and 2%) of the leaf extracts of *Azadirachta indica* (Neem), *Polygonam barbatum* (Bishkatali), *Adhatoda vasica* (Basak) and *Terminalia arjuna* (Arjun) showed statistically same effect on the growth of *Colletotrichum gloeo-*

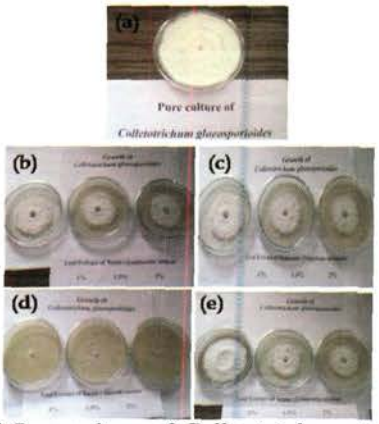

Figure 3. (a) Pure culture of *Colletotrichum gloeosporioides*. **(b)** Antifungal activities of leaf exctracts (diluted in distilled water) of *Azadirachta indica* (Neem). **(c)** *Polygonam barbatum* (Bishkatali). **(d)** *Adhatoda vasica* (Basak). **(e)** *Terminalia arjuna* (Arjun) against *Colletotrichum gloeosporioides*.

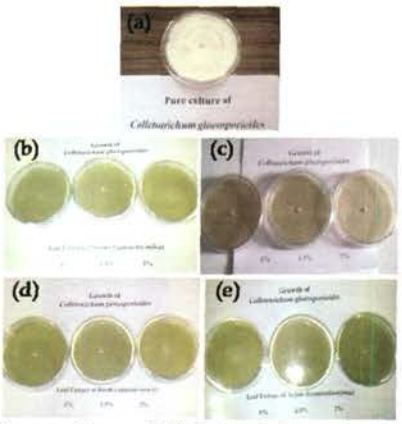

Figure 4. (a) Pure culture of *Colletotrichum gloeosporioides*. **(b)** Antifungal activities of leaf exctracts (in ethanol absolutes 95%) of *Azadirachta indica* (Neem). **(c)** *Polygonam barbatum* (Bishkatali). **(d)** *Adhatoda vasica* (Basak). **(e)** *Terminalia arjuna* (Arjun) against *Colletotrichum gloeosporioides*.

Table 3: Effect of leaf extracts of Neem (*Azadirachta indica*), Bishkatali (*Polygonam barbatum*), Basak (*Adhatoda vasica*) and Arjun (*Terminalia arjuna*) diluted in ethanol (70%) at different concentrations on the growth of *Colletotrichum gloeosporioides* and *Pestalotia theae* after 120 hr of plating

Treatments	Concentrations	Name of fungi			
		Colletotrichum gloeosporioides		*Pestalotia theae*	
		Mycelial growth (cm) Average of three replications	% Growth inhibition	Mycelial growth (cm) Average of three replications	% Growth inhibition
Azadirachta indica (Neem)	1.0%	0.60 c	100	0.20 c	97.78
	1.5%	0.00 b	100	0.06 d	99.33
	2.0%	0.00 b	100	0.00 e	100.00
Polygonam barbatum (Bishkatali)	1.0%	0.00 b	100	0.26 b	97.11
	1.5%	0.00 b	100	0.10 d	98.88
	2.0%	0.00 b	100	0.00 e	100.00
Adhatoda vasica (Basak)	1.0%	0.00 b	100	0.00 e	100.00
	1.5%	0.00 b	100	0.00 e	100.00
	2.0%	0.00 b	100	0.00 e	100.00
Terminalia arjuna (Arjun)	1.0%	0.00 b	100	0.06 d	99.33
	1.5%	0.00 b	100	0.00 e	100.00
	2.0%	0.00 b	100	0.00 e	100.00
Control	–	9.00 a	00	9.00 a	00

sporioides. All concentrations of the said plants rather than control exhibited no mycelial growth of *Colletotrichum gloeosporioides*. The results also showed that the mycelial growth of *Pestalotia theae* statistically same for 1.5% concentrations of leaf extracts of *Azadirachta indica* (Neem) and *Polygonam barbatum* (Bishkatali) and 1% concentration of *Terminalia arjuna* (Arjun). On the other hand, for 2% concentrations of *Azadirachta indica* (Neem) and *Polygonam barbatum* (Bishkatali), *Adhatoda vasica* (Basak) at all concentrations and 1.5–2% concentrations of *Terminalia arjuna* (Arjun) exhibited no mycelial growth of *Pestalotia theae*. That means all of them showed the highest growth inhibition (100%) rather than control against *Pestalotia theae*. Whereas leaf extracts of *Polygonam barbatum* (Bishkatali) for 1% concentration showed the lowest growth inhibition (97.11%) (Table 2).

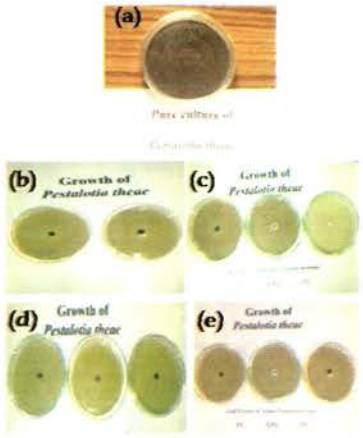

Figure 5. (a) Pure culture of *Pestalotia theae*. **(b)** Antifungal activities of leaf exctracts (diluted in ethanol 70%) of *Azadirachta indica* (Neem). **(c)** *Polygonam barbatum* (Bishkatali). **(d)** *Adhatoda vasica* (Basak). **(e)** *Terminalia arjuna* (Arjun) at different concentrations against *Pestalotia theae*.

Effect of Plant Extracts Diluted in Ethanol (70%) on the Growth of *Colletotrichum gloeosporioides* (Die-back) and *Pestalotia theae* (Grey blight)

Table 3 showed that the mycelial growth of *Colletotrichum gloeosporioides* was statistically same for the leaf extracts of *Azadirachta indica* (Neem) and *Polygonam barbatum* (Bishkatali) at their respective concentrations. The result also showed that leaf extracts of *Adhatoda vasica* (Basak) at all concentrations exhibited no mycelial growth of *Colletotrichum gloeosporioides*. Whereas the mycelial growth of *Pestalotia theae* was statistically same for 1.5% concentrations of leaf extracts of *Azadirachta indica* (Neem) and *Polygonam barbatum* (Bishkatali). On the other hand, for 2% concentrations of *Azadirachta indica* (Neem) and *Polygonam barbatum* (Bishkatali) the mycelial growth was same. Table 3 also showed that 1.5% and 2% concentrations of *Adhatoda vasica* (Basak) no mycelial growth of *Pestalotia*

theae. In this experiment the leaf extracts of *Adhatoda vasica* (Basak) at all concentrations exhibited the highest growth inhibition (100%) rather than control against *Colletotrichum gloeosporioides,* and for *Pestalotia theae* the highest growth inhibition was found 100% for 1.5% and 2% concentrations of *Adhatoda vasica* (Basak). Whereas leaf extracts of *Terminalia arjuna* (Arjun) for 1% concentration showed the lowest growth inhibition 91.11% of *Colletotrichum gloeosporioides* and 90.00% of *Pestalotia theae*.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay.[17] Archana and Abraham *et al.*[18] reported that medicinal plants have been a major source of therapeutic agents for alleviation and cure diseases. According to Khan *et al.*,[19] aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosporium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera* against *Alternaria redicina*. Total 19 plant species belonging to 14 families used by some Indians living in North America were tested for their fungicidal activity.[20] Of the species investigated by them, 9 were active against *Cladosporium cucumerinum* and 9 against *Candida albicans*. In the Zingiberaceae family, the ethanol extract of *Curcuma longa* L. and *Alpinea galanga* were also found to possess good antifungal activities against *Trichophyton longifusus*.[21] Other species of Curcuma (Zingiberaceae), *C. zedoaria* Rose, and *C. malabarica* Vel also possess antifungal activity, which supports the use of their tubers in traditional medicine for the treatment of bacterial and fungal infections.[22] In India, Sharma and Jandaik[23] found the leaves of *Azadirachta indica*, *Eucalyptus tereticornis*, *Eichhorinia crosipes*, *Tagetes erecta* and cloves of *Allium satuvum* positively active against a few test fungi. Ethanolic extracts of aerial parts and fruits of *Aglaia roxburghiana* were tested for *in vitro* antifungal activities against dermatophytes.[24] In an approach toward the development of eco-friendly antifungal compounds for controlling major foliar fungal diseases of tea, ethanol and aqueous extracts of 30 plants belonging to 20 different families collected from sub-Himalayan West Bengal (India) were tested against the fungal pathogens.[25] The antifungal potential of aqueous and ethanol extracts of eight different plant species were tested *in vitro* and *in vivo* against *Colletotrichum kahawae* in completely randomized design with three replications.[26] Examples of other antifungal crude extracts from medicinal species also included *Bauhinia racemosa* L. (Caesalpiniceae) stem bark.[27]

In the present study, it was investigated that among all concentrations of four medicinal plants diluted in all solvents, *Adhatoda vasica* (Basak) exhibited the highest effect on both fungi *viz. Colletotrichum gloeosporioides* and *Pestalotia theae* and *Polygonam barbatum* (Bishkatali) showed the lowest growth inhibition. Among the solvents, ethanol absolutes (95%) extracts showed more growth inhibition of both fungi than other leaf extracts. Thereby in this study for controlling *Colletotrichum gloeosporioides* and *Pestalotia theae* organically, all concentrations (1%, 1.5% and 2%) of leaf extracts of *Adhatoda vasica* (Basak), 2% concentration of leaf extracts of *Azadirachta indica* (Neem), *Polygonam barbatum* and *Terminalia arjuna* (Arjun) are recomendated. From this research work this is evident that all the plant extracts are potential antifungal agents against experimental tea fungi, and the effectiveness of most of the plant extracts increases proportionally with the increase in doses and decreases with time.

References

1. Ali MA, Ali M, Huq M, & Ahmed M. 1993. *In vitro* studies on fungicides against *Colletotrichum gloeosporioides* (Penz.) Sacc. – The die-back of tea. *Sri Lanka J Tea Sci* 61(1): 25–31.
2. Sana DL. 1989. *Tea Science*. Ashrafia Bio Ghar: Dhaka, 217 pp.
3. Sarma KC. 1960. Disease of tea and associated crops in North-East India. Indian Tea Association: Toklai Experimental Station, Memorandum No. 26.
4. Rattan PS. 1992. Pest and disease control in Africa. In: *Tea Cultivation to Consumption*, KC Willson & MN Clifford (Eds), Chapman & Hall: London, p. 34.
5. Derbyshire DM & Shipway MR. 1978. Control of post harvest deterioration in vegetables in the UK. *Outlines Agricul* 9: 246–252.
6. Huq M. 1995. Studies of *Colletotrichum gloeosporioides* – The Die-back pathogen on new clones and seedling of tea. *Tea J Bangladesh* 31(1&2): 23–28.
7. Huq M. 1997. Studies on isolation, culture, morphology, and identification of pathogens. Annual Report: Bangladesh Tea Research Institute, Shrimangal, 48 p.
8. Premkumar R, Nepolean P, Vidhya Pullavi R, Balamnrugan A, Jayanthi R, Kuberan T, & Beulah T. 2011. Integrated disease management of grey blight in tea. A paper presented in World Tea Science Congress, held on 22–24 November, 2011, in Tocklai Tea Research Institute, 43 p.

9. Srivastava J, Lambert J, & Vietmeyer N. 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper No. 320.

10. Clark AM & Hufford CD. 1993. Discco and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency syndrome. In: *Human Medical Agents from Plants*. American Chemical Society (ACS Symposium Series 534), Washington DC, pp. 228–241.

11. Caceres A, Jauregui E, Herrera D, & Logemann H. 1991. Plants used in Guatemala for the treatment of dermatomucosal infections: Screening of 38 plants extracts for anticandidal activity. *J Ethanopharmacol* 33: 277–283.

12. Abad MJ, Ansuategui M, & Bermejo P. 2007. Active antifungas substances from natural sources. *ARKIVOC* 7: 116–145.

13. Sinha KK, Sinha AK, Gajendra P, & Prasad G. 1993. The effect of clove and cinnamon oils on growth of and aflatoxin production by *A. flavus*. *Lett Appl Microbiol* 16: 114–117.

14. Srivastava JL. 1987. Mycotoxin problems in food in India. *Proceedings of the Joint FAO/WHOIUNDP 2nd International Conference on Mycotoxins*, Bangkok, Thailand, September 28–October 3, 1987.

15. Amadioha AC. 2002. Fungitoxic effects of extracts of *Azadirachta indica* against *Cochliobolus miyabeanus* causing brown spot disease of rice. *Arch Phytopathol Plant Prot* 35: 37–42.

16. Ravikumar P, Makari HS, & Gurumurthy H. 2007. *In Vitro* antimicrobial activity of ethanol extract of *Thevetia peruviana*. ISSN: 1579–4377.

17. Tona L, Kambu K, Ngimbi N, Cimanga K, & Vlietinck AJ. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. *J Ethnopharmacol* 61: 57–65.

18. Archana S & Abraham J. 2011. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. *J Appl Pharma Sci* 1(8): 149–152.

19. Khan TZ, Gill MA, Nasir MA, & Bukhari SAA. 1998. Antifungal properties of some plant extracts. *Pak J Phytopathol* 10: 62–65.

20. Bergeron C, Marston A, Gauthier R, & Hostettmann K. 1996. Screening of plants used by North American Indians for antifungal, bactericidal, larvicidal and molluscicidal activities. *Int J Pharmaco* 34: 233–242.

21. Khattak S, Saeed P, Ullah H, Ahmad W, & Ahmad M. 2005. Biological effects of indigenous medicinal plants *Curcuma longa* and *Alpinea galangal*. *Fitoterapia* 76: 254–257.

22. Wilson B, Abraham G, Manju VS, Mathew M, Vimala B, Sundaresan S, & Nambisan B. 2005. Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. *J Ethopharmacol* 99: 147–151.

23. Sharma VN & Jandaik CL. 1994. Effect of some plant materials in controlling different moulds in *Agaric us bisporus* (Lang) Imb. *Indian J Mycol Plant Pathol* 24: 183–185.

24. Janaki S, Viswanathan S, Vijayasekaran V, Parvathy SS, Ramanujam TR, Prathiba D, & Rao KR. 1998. The effect of *Aglaia roxburghiana* (W. and A.) miq. Var. beddomei on experimentally induced ulcers. *Indian J Pharma Sci* 60: 402–404.

25. Saha D, Dasgupta S, & Saha A. 2005. Antifungal activity of some plant extracts against fungal pathogens of tea (*Camellia sinensis*). *Pharmaceutical Biol* 43: 87–91.

26. Abera A, Lemessa F, & Muleta D. 2011. The antifungal activity some medicinal plants against Coffee Berry Disease caused by *Colletotrichum kahawae*. *Int J Agri Res* 6: 268–279.

27. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, & Mazumder UK, *et al.* 2005. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. *Brazilian J Med Biol Res* 38: 1015–1024.