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Research Article

STUDIES ON ENTOMOPATHOGENIC FUNGUS *ASCHERSONIA ALEYRODIS* INFECTIOUS TO SCALE INSECT PEST, *SAISETTIA FORMICARII* OF TEA, *CAMELLIA SINENSIS* (L). O.KUNTZE IN ASSAM

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ABSTRACT
Tea, *Camellia sinensis* (L).O.Kuntze is an economically important plantation crop in India which support livelihood of about 1.5 million people. The total annual production of tea is about 1200 million kg and 75 percent of which is contributed by tea industry of North East India. Tea agro ecosystem is unique and mimics forest ecosystem in its full bearing. Leguminous shade trees are planted in between tea which provides shade and shelter to tea plants underneath. Cultivated tea plants are pruned at predetermined height to encourage vegetative growth. Scale insect are considered one of the economically important pests of tea, *Camellia sinensis* (L).O.Kuntze in North East India (Lat-24^{o}-28^{o}Nto Lon-88^{o}–95^{o}E) having limited distribution. Although numerous natural enemies of scale insects of tea are reported from North East India no reliable bio intensive IPM approach is available. As a result use of pesticides remained as the dominant means of controlling the menacing pests. The fungus, *Aschersonia* spp probably the earliest known entomopathogenic fungus reported on scale insects. The fungus was found to occur naturally on several species of *Saisettia* throughout North East India and kept the population of scale insect *Saisettia spp* at bayin certain localities. The scale insect usually infests young tea stems and ventral surface of leaf and later spreads to other parts of the plant. A species of black ants, *Technomyrmes albipes* Smith and red ant, *Oecophylla smaragdina* Feb remained associated with colonies of scale insects infected by *Aschersonia* spp. Ants gradually disappear once the fungal infections spreads with time and caused heavy mortality of scale insects within the colony. Ants are not found to be susceptible to fungal infection. Tea cultivation in North East India is intensive and agronomic practices like pruning, skiffing, use of agrochemicals, winter cultural practices etc carried out on tea exert considerable inhibitory influence on growth of *A.aleyrodis*. Conidia of *A.alerodis* are produced in large quantities in gelatinous mass which can be carried away by insects coming across or transmitted by raindrops during rains. Conidia are hyaline, spindle shaped, small (12-8X4-5 u) and germinate by producing one or two germ tubes. Most conidia germinate by production of capilliconidia, a special structure which is known to aid in transmission of propagulae in entomopathogenic fungi. Perfect stage or teleomorph are rarely formed under field conditions but can readily be induced by incubating tea twigs bearing stromatic fructification under high humidity in laboratory. The present work deals with the interaction of *A.alerodis* with complex agroclimatic conditions prevailing in tea ecosystem in Assam and to explore its potential to develop as a biocontrol agent.

***Keywords*:** *Aschersonia Aleyrodis, Camellia Sinensis, Saisettia, Biocontrol, Scale Insect*

INTRODUCTION

Scale insects are serious pests of tea, *Camellia sinensis* (L).O. Kuntze in North East India (Das, 1959; 1960; 1965). Watt (1898) made pioneering studies and reported ten species comprising four species of Diaspididae and six species of Coccidae. Later Watt and Mann (1903) recorded fifteen species of which six belonged to Diaspididae, six to Coccidae and one each to *Asterolecinidae, pseudococcidae and Lacciferidae*.

Subsequently, Das (1959) made a comprehensive study on scale insects in tea in North east India and their natural enemy complex. Das and Ganguli (1961) mentioned five species of *Saisettia (S.coffeae; S.nigra*; *S.oleae; S.watti* and *S.formicarri)* which occur on tea throughout North East India while *S.*

formicarii was found to occur predominantly on tea in Assam. Forty four species of scale insects have been reported on tea in North East India and is shown in table I.

Reports of damage to Tea plants due to scale insects continue to cause worry to planters in Upper Assam and West Bengal (Rahman *et al.,* 2007).

A new type of scale insects was reported to cause damage in tea in Upper Assam however, the specific identity of the scale insect was not ascertained. Although numerous natural enemies of scale insects of tea are reported from North East India (Das, 1974; 1979; Debnath, 1991) no reliable bio intensive IPM approach is available (Muraleedharan and Silvasundaram, 2002).

As a result use of pesticides remained as the dominant means of controlling the menacing pests. The resurgence of scale insects was largely attributed to the side effect of plant protection chemicals used for the suppression of other insects like red spider mites, helopeltis, looper, thrips jassids and redslug. Natural occurrence of fungal pathogens of scale insects was recorded in the past from tea ecosystem (Debnath, 1985; 1986; 1987; 1991; Debnath *et al.,* 2001).

Studies on the impact of native entomopathogenic fungus and their prospects of biocontrol are poorly understood.

The fungus *Aschersonia* (teleomorph: *Hypocrella libera*) probably the earliest known entomopathogenic fungus belonging to deuteromycetes with over 78 described species (Petch, 1921; Sutton, 1980, Chaverri *et al.,* 2008).

The fungus was recognised as effective biocontrol agents in Florida for control of *Aleyrodes cirti*as early as Fawcett, (1908).

The classical control of citrus white fly *Dialeurodis citri* in USSR was achieved by introducing *Aschersonia* species from India, China, Japan, vietnum, USA and Cuba. The citrus white fly was accidently imported with no natural enemies in USSR in 1957.

Protsenko (1967) reported that 80 percent larval mortality of white fly was obtained with foliar spray of conidia of *Aschersonia* in Azerbaijan. Moreover, the introduced fungus adapted well and spread to new areas of citrus plantations.

Uchida (1970) observed that fungicide application reduces effectiveness of *Aschersonia* used for control of *D.citri* in Japan. Solovey and Koltsov (1976, 1982) observed that a Cuban isolate of *Aschersonia* provided 83 percent mortality of orange white fly in USSR. Ramakar (1983) recorded biocontrol of cucumber white fly in green house in Holland by *Aschersonia* along with a parasitic fly *Encarsia spp.* Ellis *et al.,* (2002) studied pathogenecity of *Aschersonia* spp. against whiteflies *Bemisia argentifolia* and *Trileurodis vaporariorum* in green house in Holland. Evan (1994) reported occurrence of capilliconidia of *Aschersonia* spp.

Iren and Soran (1975) studied mass production of the fungus *Aschersonia aleyrodis*, a pathogenic agent for *Dialeurodes citri*. Muma (1969) studied biological control of insects pest and mites of citrus by *Aschersonia* in Florida.

Oho (1967) reported possible utilization of *Aschersonia aleyrodis* for control of *Dialeurodes citri* insect pests in Japan. Prilepskaya *et al.,* (1975) studied *Aschersonia* to control white fly in Russia. Prinak and Chizhik (1975) explored the possible use of *Aschersoniaaleyrodis* for the control of glass house white fly *Trialeurodes vaporarium.*

Ramaker and Samson (1984) studied *Aschersonia aleyrodis*, a fungal pathogen of white fly and explored its potential as biological insecticide in glass houses. Rolts and Fawcet (1908) made pioneering studies on fungus diseases of scale insects and white fly in US.

Solovei and Sogoian (1982) studied fungus *Aschersonia* against the glass house white fly, biological control of *Trialeurodes vaporariorum* (USSR).

Spasova (1974, 1980) investigated the use of the entomopathogenic fungus *Aschersonia* against *Trialeurodes vaporariorum* in USSR.

Spassova *et al.,* (1980) studied pathogenecity of various species of fungi belonging to the genera *Aschersonia* to the larvae of glass house white fly (*Trialeurodes vaporariorum* West Wood) on tomato and cucumber. Stolyarova (1972) studied *Aschersonia* against the Citrus white fly in USSR.

Table I: Scale Insects Occurring on Tea Plants in North East India

Species	Plant Part Affected	Nature of Damage	Distribution
Family: Coccidae			
1.*Saissetia coffeae* (Wlk)	Young shoots, leaf	Severely affected shoots die back	All over NE India
2.*S.nigra* (Nietner)	Stems	Not prominent	Sporadic
3.*S.oleae (*Barnard)	Stems and leaf	Not prominent	Assam
4.*S.formicarii* (Green)	Stems and leaf	Severely affected stem remained stunted, die back	All over NE India
5.*S.watti* (Green)	Stems	Affects stem	Sporadic
6.*CoccusdiscrepensI*(Green)	-	Not prominent	Tocklai, Assam
7.*C.hispiridium* L	Stem and leaf	Affects stem and leaf. Often cause serious damage to health of tea.	All over NE India
8.*C.viridis* (Green)	Leaf,stem	Colonise leaf midrib, stems	Sporadic
9.*Eriochiton theae* (Green)	Leaf and stem	Affected leaf turn yellow, drop off, infested branch debilitated	Darjeeling, Dooars and Assam
10. *Eucalymnatus tessellates*	Leaf	Not prominent damage	Assam
11.*Ceroplastids rubens(*Maskell)	Branch,Primares	Severely infested branch die back	Darjeeling, Dooars and Assam
12.*C.floridens* (Combstock)	Stem and leaf	Not prominent damage	Assam
13.*Ceroplastids chiton* Green	Twigs	Not prominent damage	Assam
14.*C.cajani* (Maskell)	Twigs	Not prominent damage	Assam
15.*Vinsonia stellifera*(Westwod)	Leaf	No prominent damage.	Assam
16.*Chloropulvunaria floccifera* (West wod)	Leaf	No prominent damage	Darjeeling
17.*Hemiberlesia rapax*(Conbstock)	Leaf Petiol	Induces defoliation	Darjeeling
18.*H.lataniae* (Signorat)	Leaf	Induces defoliation	Dooars and Assam
19.*Morganella longispina* (Morgan)	Branch	Causes die back of branches	Assam
20.*Lindingaspis ferrisi* (McKenzie)	Stem	Severely infested brancj die back	Borbhetta, Assam
21.*Chrysomphalus aonidium* Ashmead	Leaf	Affected leaf get detached, plant become	Assam, Dooars and

		weak.	Darjeeling
22.*C.pinnulifer* (Maskell)	Leaf	Sporadic	Dooars
23.*Aoniediella aurantii* (Maskell)	Leaf	Tea plant in nursery most susceptible.	Dooars and Assam
24.*Pseudaoinidia duplex*(Ckll)	Stem	Not prominent	Tocklai, Assam
25.*P.trilobitiformis*	Leaf	Not Prominent	Tocklai, Assam
26.*Clavispis spp*	Stem	Branches dieback in severe infestation	Assam
27.*Abgrallaspis cyanophyll* (Signoret)	Leaf	Not prominent	Assam
28.*Abgrallaspis pictor* (Williams)	Stem	Not Prominent	Darjeeling
29.*Aspidiotus destructor*(Signoret)	Leaf	No prominent damage	Dooars and Assam
30.*A.spinosus*(Signoret)	Stem	No prominent damage	Assam
TRIBE: DIASPIDINAE:			
1.*Fiorinia theae* (Green)	Leaf and stem	Leaf turn yellowish, branch die back	Assam and Dooars
2.*Pinnaspis theaeI* (Maskell)	Leaf and stem	Leaf defoliation and die back	Darjeeling and rarely in Dooars
3.*Velataspis serrulata* Ganguli	Leaf and stem	Causes serious damage to seedling tea.	Darjeeling
4.*Phenacaspis manni* (Green)	Stem and leaf	Stem swelling at the point of feeding, die back.	Darjeeling
5.*Phenacaspis spp*	Leaf	Leaf defoliatin	Assam
6.*Andaspis dasi* Williams	Stem	Stem die back	Darjeeling
7.*Parlotoria proteus* (Curtis)	Leaf	Leaf discolouration.	Darjeeling
8.*Lepidosaphes spp*	Leaf	Leaf chlorosis	Assam
9.*Chionaspis sepereta* Green	Leaf	No prominent damage	Darjeeling
10.*Nipaecous vastator* (Maskell)	Stem and leaf	Leaf curl wards. Stem die back	Assam
11.*Pseudococcus spp*	Roots	Affects root tissue growth stunted	Darjeeling, Assam
12.*Crisiococcus spp*	Roots	Affected root show poor growth	Darjeeling
13.*Rhizoecus spp*	Roots	Restricted growth of affected young tea.	Darjeeling
14.*Pseudoaladiopsis pentagona*	Stems and collar regions	Affected stem covered with white growth of scale insects	Assam and Dooars

Taxonomic Consideration

Chaverri *et al.,* (2008) studied taxonomy of *Aschersonia* and its perfect stages Hypocrea. Petch (1921) classified the genus in to two sub genera based on the presence and absence of paraphyses, *Euaschersonia* bear paraphyses within pycnidia while subgenera *Leprieuria* lacks paraphyses. *A.alerodis* was first reported by Webber (1894) as *A.tahitensis* Mont on white fly. However, later Patouillard stated it to be a distinct species. Petch (1921) concluded that *Hypocrella libra* is the perithecial stage or perfect stage of *A.aleyrodis*. Description of *Aschersonia* as per Main (1961) is given below:

"Stromata flattened pulvinate to scutate 0.5-5 mm wide, usually 1-3 mm surrounded by a thin hypothallus 1 mm wide white to ochraceous, usually more or less covered by a copious mass of spores, orange when fresh, with few to round elongated pycnidial orifices often radially arranged. Pycnidia spherical to irregular. Pycnidiospores fusoid acute at both ends, hyaline, frequently guttulate. Paraphyses filiform, variable 50-150µ long, hyaline."

The present investigation was carried out to reassess the natural occurrence of *Aschersonia aleyrodis* on tea ecosystem in Assam and examine the prospects for practical application under field conditions for suppression of scale insect pests of tea in Assam.

MATERIALS AND METHODS

Study Area

Site 1: The survey was carried out on tea experimental plot located at Tocklai Tea Research Institute, Jorhat (Lat 26^0 43' 49" N- Lon 94^0 13' 42"E. The area planted in 1959 with mixed varieties of tea and later in filled with known cultivars. A total of 1403 no of the JTCL cultivars were planted during 1959 in the area. The soil is sandy loam, pH 4.91, organic carbon 1.64 percent and available Nitrogen and Potash 120 and 100 ppm respectively.

Site 2: A total of 1399 tea plants were planted including 411 plants of tissue culture raised plants during 1998 and the rest area was planted with tea cultivar 317/2-8 known as Darjeeling flavoury clone during 1959. The entire area was moderately infected by scale insects particularly *Saisettia* spp and was selected for monitoring the incidence of scale insects and associated entomopathogenic fungus *Aschersonia spp*.

Observation was carried out during the year of prune i.e. 2012 and site of attack, height from the ground, percent tea plants infested by scale insects and associated entomogenous fungus *Aschersonia* in different stages of parasitisation, percent tea plants associated with *Aschersonia* without any visible presence of active scale insects individual, presence of ants nest and activity, Sooty mold. The observations for both sites 1 and 2 were recorded and the mean value shown in table 1.

Site 3: This area was planted during 2006 and received agro inputs as per conventional practice. The area was planted with experimental tea cultivars 30 plants each of 662, 657, 663, 656, TV-1, S3A3, 658, 659, Tinali 17. Besides a total of 690 plants of mixed cultivars were also planted. The soil registered pH 4.91, organic carbon 1.64 and available Nitrogen and Potash 120 and 98 ppm respectively. The observation was confined to 10-45cm of bush height from ground. The survey was undertaken in young tea area on 960 plants and the observation recorded shown in table 2.

Germination of Conidia of A. aleyrodis on Leaf Leachate and Stem Washing

Germination of conidia of A. aleyrodistes on leaf leachtes and stem washings was assessed as per Godfrey and Clements, 1978. Briefly ten mature leaves (approximate 1000 cm^2) and five tea stem bits 2 cm length of pencil thickness were collected from two different field and were dipped in 100 ml sterile distilled water separately for 20 minutes as per with little modification. Leaching were filtered and the filtrate were use for germination test against *A. Alerodis* as per Fransen 1987. The result of germination test is shown in table 3.

Isolation and Cultural Characteristics

Tea stem bearing fresh fructification of *Aschersoni*a were collected from the field and washed thoroughly with sterile distt water and then treated with 50% aqueous ethyal alcohol for 60 seconds and washed again several times with sterile distt. water. These stem bits were incubated under moist petriplate as per Keyworth (1951) at 25-30^0 C for 5 days. Spore mass started oozed out from the opening of ostiole and

accumulating in a gelatinous glistening mass of variable shape. These spore masses were transferred aseptically in to 2 ml of Czapek dox broth amended with 0. 1 Percent yeast extract and 100 ppm of Chloramphenicol on 10 cm sterile petriplates. A molten Czapek dox agar medium is poured and plate is incubated at 30^0 C. Observation on colony growth and sporulation following incubation were recorded and shown in table 4.

Sporulating Ability of A. Aleyrodis

Tea stem bits of 3 pencil thickness (1 cm diameter) were collected from the field during March from the infected area bearing fructification of *Aschersonia* (stromatic fructification). Four stems of different sizes were collected and incubated under moist condition inside 15 cm petridish lined with filter paper (Keyworth, 1951). The observation was made at periodic interval and final observation on pycnidial opening based on oozed out conidial mass was recorded in table 4.

RESULTS AND DISCUSSION

Table 1: Showing Natural Incidence of *Saissettia* and Entomopathogenic Fungus *Aschersonia* on Tea Plants in Site I and Site II

Parameters	Site 1	Site 2
Bushes examined	2802	960
Percent Tea plants infested by scale insects and *Aschersonia*	44	22.97
Mean percent tea plants with *Aschersonia* fructification with no visible presence of scale insect individual and ants.	14	3
Tea bushes with ants and their activity and no visible presence of scale insect	71	80
Percentage of bushes with Presence of sooty molds	Nil	2

It was evident from table 1 that scale insect infestations were confined to height of pruning level which varied depending on the age of plants. Young plants are pruned at lower height than the old tea plants. Stems and forks created by cross branching and leaves of Young tea plants are found to be infested by scale insects at a height of 10 cm of ground level up to 45 cm of bush height in both site I and II. Infestation of stems and leaves of mature tea was observed from 20 cm to 65 cm of bush height in site I. *Aschersonia* fructificatons were observed on both surfaces of leaves (plate: 1.2 and 3) and on tender stems (plate: 4 and 5) and on mature stems (plate 6). There were 44 and 23 percent tea plants infested by scale insects and *Aschersonia* with different stages of parasitisation in site I and II respectively. The mean percent infestation of Aschersonia with no visible presence of Scale insects individual in site I and II were 14 and 3 percent respectively. Ants activity was found to be prevalent on bushes in both the sites (site I, site II) with no visible presence of scale insects. Sooty molds were observed in site II on 19 bushees. It was also evident that colonies of young scale insects were infested individually by *Aschersonia* on tea stems or leaves. Ants were found to attend scale insects colony (Plate 7). Fructification of *Aschersonia* develop into irregular mass (Plate 8) with small opening through which spore mass ooze out.

Table 2: Showing Natural Incidence of Scale Insect Infestation in Young Tea Cultivars in Site 3

Tea Cultivars	No of Bushes	Percent Tea Cultivars with *Saisettia* Spp and *Aschersonia*
662	30	36.66
657	30	13.33
663	30	30.00
656	30	16.66
TV-1	30	26.66
S3A3	30	10.00
658	30	10.00
659	30	3.33
Tinali 17	30	56.00
Mixed Cultivars	690	27.10

It was evident from the table 2 that tea cultivar varied depending on their susceptibility to scale insects Tinali 17 was found to be highly susceptible while tea cultivar 659 was least susceptible to scale insects infestation. There were 56 and 3 percent infestation of scale insects on Tinali 17 and 659 respectively.

Table 3: Effect of Leaf and Stem Washing on Germination of *Aschersonia Aleyrodis*

Treatment	% Germination	Length of Germ Tube	Remarks
Site – I Leaf washing	95	3 – 12 µ	Capilliconidia seen
Site – II Leaf washing	90	3- 12 µ	-do-
Stem washing	98	3- 12 µ	-do-
Control distilled water	80	3- 10 µ	

Spores are spindle shaped (Plate 9), aseptate, and germinate after a long lag phase (plate 10). Spore swell up germinated after producing a small germ tube. In some cases a round structure protrudes from one end of spore not resembling a typical germ tube.

This is probably an initiation of capilliconidia in *Aschersonia* which occur in various shape and sizes (Plate 11), occurrence of capilliconidia and its biological significance was studied by Evans (1994). Branched capilliconidia were seen during germinaton of conidia.

The germ tube elongation in few spores almost equal to the length of spores within 26 hours in PDA medium.

It was evident from table 3 that increased germination of spores was observed in leaf washings collected from site I and site II over untreated control. Leaf washings from site I induced higher germination of spores. Stem washings (1:10) showed higher germination. Higher germination in leaf and stem washings may be attributed to effect of concentration of nutrient presents in the leachates. Spore germinated primarily by single germ tube from one end of conidia however bipolar germination also observed (Plate: 10).

Table 4: No of Pycnidia and Development of Spore Mass of *A. alerodis* on Tea Stem Surface under Moist Chamber

Stem Bits	Length of Stem Bits	No of Pycnidia	No of Pycnidial Opening	Mean Sporulating Ability/Pycnidial Opening
1	9	56	34	$5x10^6$ Conidia
2	9.2	46	15	
3	10	20	10	
4	9	84	35	

The spore oozed out upon hydration as globose mass, sometimes long spiral thread like structures or cirri (Plate 12-15).

Pycnidial mass aggregates at the opening of ostiole. At a later stage yellow spore mass dry up accompanied by formation of flask shaped ostiol from the stromatic surface. It takes about a month to develop under 16 hour light and 8 hours dark period. Microscopic examination revealed presence of typical ascus and ascospors with the stroma.

Cultural Chracteristics of A. Aleyrosdis

Colony slow growing dull white later becoming yellowish in colour. The radial growth of the colony is extremely slow reaching 5mm in 7 days (Plate 12).

Surface of colony is flat at first and later covered with appearance of abundant yellowish spore mass which are gelatinous and remained aggregated.

Plate: 1

Plate: 2

Plate: 3

Plate: 4

Plate: 5

Plate: 6

Plate: 7

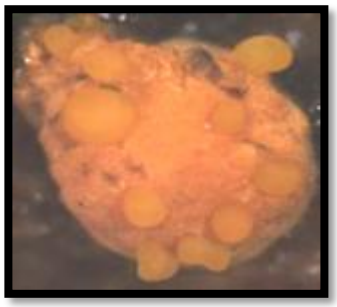

Plate: 8

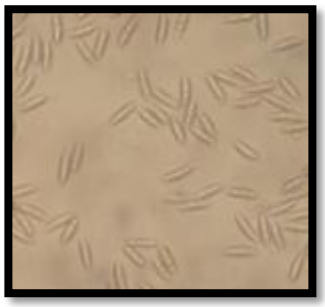

Plate: 9

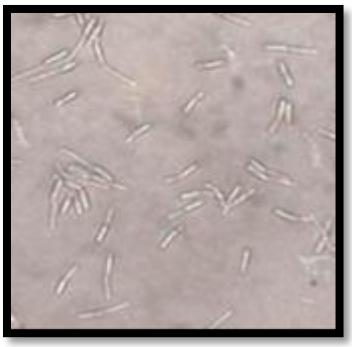

Plate: 10

Plate: 11

Plate: 12

Plates: (1-12): 1. Young Larvae of Scale Insect Parasitized by *A. Aleyrodis* on Ventral Surface of Leaf; 2. Do – On Dorsal Surface; 3. Stromata of *A. Alerodis* on Leaf Midrib with Slimy Spore Mass; 4. Stromata of *A. Alerodis* on Small Tea Stem with Slimy Spore Mass; 5. Stromata of *A. Aleyrodis* on Tea Stem; 6. Young Colony of Scale Insect, *S. Formicarii* Parasitized by *A. Aleyrodis*; 6. Colonies of Scale Insect Parasitized by *A. Aleyrodis* on Tea Stem; 7. Ants Association with Scale Insect Colony on Tea Stem; 8. Spore Exudation Pattern from Tromata (Round Spore Mass); 9. Typical Spindle Shaped Unicellular Conidia of *A. Aleyrodis;* 10. Germination of Conidia of *A. Aleyrodis; 11.* Capiliconidia of *A. Aleyrodis*; 12. Cultural Characteristics of *A. Aleyrodis* (Radial Growth)

Conclusion

Tea ecosystem in North East India provides ideal habitat for sustaining large diversity of insects species, microbial resources and natural enemy complex. *Aschersonia* spp is a native entomopathogenic fungus which regulates scale insect species where ants act as non host vector. The present study clearly demonstrated the role of entomopathogenic fungus *Aschersonia aleyrodis* for regulation of scale insects population in tea ecosystem in North east India. Ability of *Aschersonia* spp to grow in artificial culture made it suitable for mass production for practical application. There is however a need to study the species diversity of *Aschersonia* throughout North East India. *Aschersonia* may be developed and used as a biological control agents as well as indicator of sustainability in conventional and organically grown tea. *Aschersonia* has immense potential to develop as augumentative classical control of scale insect in tea plantation.

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