whose AE data are analysed and presented in the present work is a part of the set of beams considered in the study using tri-linear model[18]. The size-independent fracture energy calculated using the tri-linear model[18] was 219.6 N/m, which is fairly close to the value estimated in the present study. This confirms that the above method, although approximate, gives fairly reasonable values of size-independent fracture energy.

Thus the present study reaffirms not only the versatility of the AE technique to visualize FPZ formation in real time, but also its usefulness in estimating the size of FPZ and consequently the size-independent fracture energy. The FPZ estimated using the AE technique, having higher density of events in the interior and lesser density surrounding it, substantiates the assumption made in the BEM regarding FPZ. The FPZ length estimated using AE technique is useful in calculating the true fracture energy. The true fracture energy calculated from the present method, which could be viewed as an approximate method, is 216.7 N/m. This is in good agreement with the size-independent fracture energy of 219.6 N/m estimated using the tri-linear model[18].

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Colonization and antagonistic activity of entomopathogenic *Aspergillus* sp. against tea termite (*Microcerotermes beesoni* Snyder)

Tea (*Camellia sinensis* L.) is widely planted in the tropical and subtropical zones, including India, China, Vietnam, Japan and Sri Lanka. India produces 35% of the world's tea, and its 400,000 ha land in the northeast region is under tea cultivation[1]. However, despite the availability of improved technology, nutrition and fertility management[2], biotic stresses like insect pests and diseases limit the productivity of tea[3,4], resulting in 11–55% annual loss in yield[5]. Termites include more than 2600 species around the world[6], but only few (70–80 species) are considered of economic importance. In India, there are eight species of termites that cause damage to tea plants, out of which *Microcerotermes beesoni* Snyder, *M. obesi* Holmgern and *Odontotermes feae* have been identified and confirmed by the Zoological Survey of India, Kolkata[7] from the Barak valley, Assam. There has been heavy usage of organo-synthetic pesticides since 1950s against termites, leading to rapid conversion of innocuous species into pests, development of resistance, and undesirable pesticide residues in tea. As a result, pesticide residues have become a major problem for the tea industry[5]. This raises an urgent need for the use of biocontrol measures for termite control. In the Barak valley, infestation of termite is the predominant cause that limits tea production. Therefore, the present work was undertaken with an objective to isolate the entomopathogenic microorganisms and study their biocontrol/colonization ability against tea termites infesting live wood tea plants of the tea gardens in Barak valley.

A dark chamber was designed to provide a suitable environment for the termites in laboratory condition. It was prepared by wrapping black paper inside a box on all sides. Normal or tap water of about 50–100 ml was sprayed over the nest. The colonies were maintained at 27 ± 2°C and 70–80% relative humidity by keeping in a cool, dark area. A piece of nest collected from the tea garden was gently crushed and termites were transferred and trapped with the help of smooth fibre brush. The tea termites were identified as *M. beesoni* Snyder on the basis of morphological characteristics, mainly mandibular region and antennal part.

Soil samples were collected from tea gardens for the isolation of microorganisms. One gram of soil sample was suspended in 10 ml sterile water, vigorously mixed and left overnight. Then 200 µl of this suspension was transferred to potato dextrose agar (PDA) plates for isolation of fungus. The plates were incubated in inverted position at 30°C for 48 h. The pure culture of isolates was maintained in PDA vials and stored at 4°C. Conidial suspensions were prepared as inoculum by scraping the fungal culture surface with a sterile *L*-shaped stick (cell spreader). The conidial clumps were suspended in distilled water. The suspensions were vortexed for 5 min to dissociate the conidial clumps, and filtered through a layer of cloth to remove the clumps and mycelial debris. Concentration of the suspensions was determined using a Neubauer hemocytometer, and confirmed by enumerating viable counts on PDA. Conidial suspensions were suitably diluted depending on the experiment, stored at 4°C and were used on the same day or the day after preparation.

Most of the research on termite biological control has followed the concepts of classical biological control of other insect pests using microbial pathogens[8,9]. Due to the cryptic habitat and social organization of termites, biological control has to be modified from strategies used in agricultural crops. Earlier, inundative method was used for termite species with a central nest structure, one-piece nesting type, or intermediate nesting type[10–14]. However, some technical limitations were encountered and colony control was found to be inconsistent. Unfortunately, the inundate method is not realistic for termite species with a diffuse nest structure (extended nesting type), such as subterranean termites, because only a small fraction of the colony is accessible. Occurrence of an epizootic in subterranean termite species relies upon transmission of the pathogenic agent among all individuals in the colony, which is difficult due to avoidance of the treated areas by healthy individuals[15]. Such treatments could use pathogens as a repellent for temporary protection of the treated area, but are not likely to achieve colony-level control. Therefore, alternative protocols have been deemed necessary to introduce pathogens into a subterranean termite colony. Another approach of 'baiting' has also been considered[16–18], but the development of a stable and non-repellent formulation remains a problem. Despite efforts to screen for virulent strains of pathogenic agents, the delivery of sufficient inoculums to a subterranean termite colony remains an unsolved problem[19]. Therefore, 'trap and treat' method[20] has been utilized in the present work for the screening of antagonistic activity of all the fungal isolates against termites. Briefly, worker termites were collected from the colony and treated with a virulent entomopathogen, followed by release into their original nest to contaminate the rest of the colony. However, it is difficult to inoculate enough individuals simultaneously to trigger an epizootic within the colony[21]. Therefore, the method was modified by making the termites sensitive as they were treated in petri dishes after being trapped from the nests (*in vitro*) as suggested[7] (Figure 1 *b*). The mortality of termites was accessed in triplicates, with 20 workers in each colony, and standard error of mean was calculated. The statistical significance of these measures was assessed using analysis of variance technique to compare the treatment means at 5% level of least significant difference (LSD).

Several bacteria (not elaborated here) and fungi were isolated from soil samples. Fungal population was dominated by species of *Fusarium*, *Penicillium*, *Aspergillus*, *Rhizopus* and *Cladosporium*. All the isolates were screened for their entamopathogenic activity against *M. beesoni* Snyder. One of the isolates, *Aspergillus* sp. TK (Figure 1 *a* and *c*) was found to have excellent colonizing ability on the termites (Figure 1 *d* and *e*). *Aspergillus* sp. TK was identified up to the genus level on the basis of macro- and micro-morphological characteristics[22]. The isolate was fast-growing and covered the PDA plate within 4–5 days at 28°C. The colonies were light green in colour on PDA. The conidial head typical to aspergilli was observed to be uniseriate and radiate type with globose vesicle (Figure 1 *a* and *c*).

Under direct contact with *Aspergillus* sp. TK in petri dishes, *M. beesoni* Snyder exhibited significant mortality when compared with the control at the concentration of 3.69×10^7 conidia/ml, where 50% mortality was observed within 48 h, while all of the worker populations died within 5 days after treatment (Figure 2). The mortality rate at the concentration of 6×10^8 conidia/ml and $>10^9$ was recorded 60% after 48 h. The rate of mortality was relatively less at concentrations below 10^6 conidia/ml. However, there was no mortality in the control up to the duration of experiments.

The role of *Aspergillus* sp. TK in mortality of *M. beesoni* Snyder was ascertained in accordance with Koch's postulates. Invariably, it was observed that (i) *Aspergillus* sp. TK was present in every case of the infection but absent from uninoculated healthy termites (control); (ii) *Aspergillus* sp. TK was isolated and grown in a pure culture; (iii) the

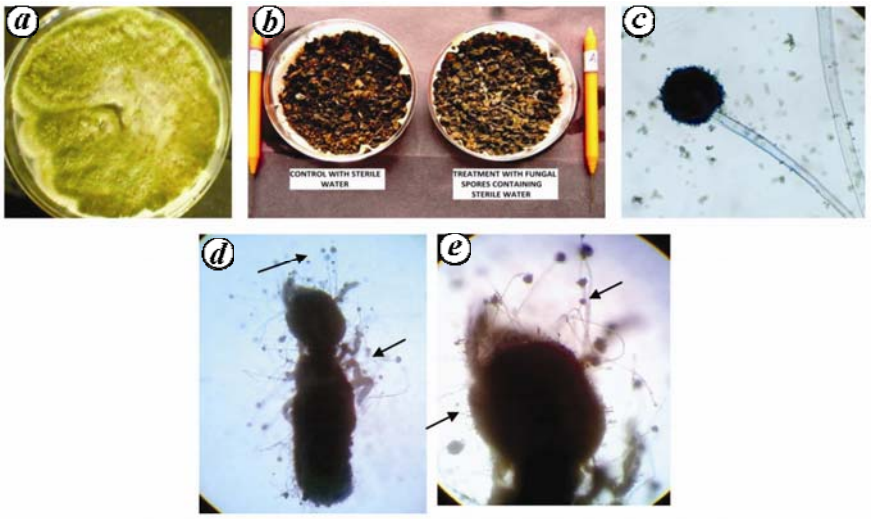

Figure 1. ***a***, Growth characteristic of *Aspergillus* sp. TK on PDA plate. ***b***, Maintenance of termite colonies and screening of antagonistic activity of *Aspergillus* sp. TK against tea termites. ***c***, Conidium of *Aspergillus* sp. TK (1000×). ***d***, Colonization of *Aspergillus* sp. TK on the surface of dead termite (200×). ***e***, Profusely colonized mandibular region of dead *Microcerotermes beesoni* Snyder (400×).

termites got profusely colonized and infected when the isolated *Aspergillus* sp. TK was inoculated into healthy workers, (iv) and *Aspergillus* sp. TK was re-isolated again from the dead termites. The effect of cellulose treatment on mortality rate of termites was also studied. The worker termites were maintained on either sawdust or crushed termite nest and inoculated with *Aspergillus* sp. TK. In both the trials, treatment of cellulose improved mortality rate. When infected with *Aspergillus* sp. TK, the mortality of termites was 50% better after 4 days in colonies maintained on cellulose-amended sawdust, compared to those maintained with sawdust only. During the incubation of four days, the difference in mortality was significant between cellulose amended and non-amended trials in sawdust (at 5% level of LSD). Similarly, the difference in mortality was significant between cellulose amended and non-amended trials in crushed termite nest for three days (at 5% level of LSD). In fact, 100% mortality was observed just after 3 days in the colonies of workers maintained in sterilized and crushed termite nest amended with cellulose compared to crushed termite nest only, when infected with *Aspergillus* sp. TK (Figure 3). Further, the mortality rate was relatively higher on crushed termite nest compared to sawdust, the reason for which remains unexplained. Also, pairwise comparisons between mortality rate on sawdust and crushed termite nest in cellulose amended/non-amended trials were made using one-way ANOVA (see supplementary data online), where all the means were found to be significantly different for each day. The dose of inoculum was maintained at 3.5×10^7 conidia/ml.

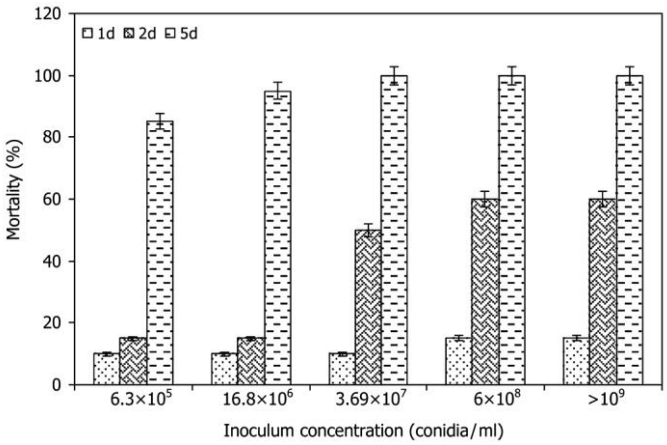

Figure 2. Concentration mortality response of *Aspergillus* sp. TK against *M. beesoni* Snyder in petri dishes. Bars represent standard error of mean calculated from three colonies with 20 workers in each colony.

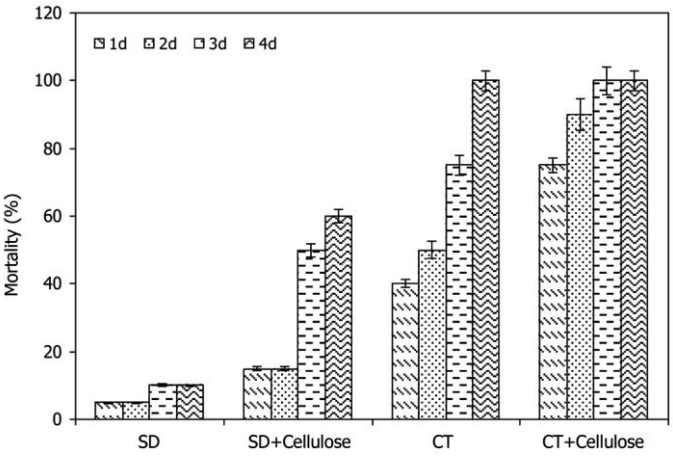

Figure 3. Effect of *Aspergillus* sp. TK against *M. beesoni* Snyder with untreated sawdust (SD) or crushed termite nest (CT) along with treated cellulose bait. Bars represent standard error of mean calculated from three colonies with 20 workers in each colony.

It has been reported earlier that cellulose bait improves the effectiveness of *Metarhizium anisopliae* as a microbial control of termites[18]. In the present study, a similar effect was observed for *Aspergillus* sp. There are few reports available, where *Aspergillus* sp. have been studied for its entomopathogenic activity in arthropods other than termites. For example, *A. flavus* has been reported to cause mortality in *Coptotermes formosanus*[23]. Similarly, *A. flavus* Link was identified as a fungal pathogen of *Reticulotermes* species[24]. In fact, *A. ochraceus*, *A. kanagawaensis* and *A. sulphurous* have been reported to cause 80% mortality in second-stage larvae of *Aedes fluviatilis* and *Culex quinquefasciatus*[25]. Recently, *A. terreus* was reported as entomopathogenic fungi for the control of ticks (*Hyalomma anatolicum anatolicum*)[26]. However, here we report the antagonistic potential of *Aspergillus* sp. against tea termites. In spite of the effectiveness of entomopathogenic microorganisms, the defence mechanisms of termites remain a major concern in their biological control. Most of the microbes fail to deliver good results because the termites have mechanisms that prevent infection from spreading in the colony. These preventive strategies reduce the survival of entomopathogens in a viable colony of termites. This behaviour forms the basis of the hypothesis that the homeostatic colony environment of social insects tends to reduce the virulence of potential parasites[21]. In the present study, the inoculation resulted in profuse colonization on termite surface with 100% mortality rate within 5 days. Conclusively, *Aspergillus* sp. TK was found to colonize and kill *M. beesoni* Snyder efficiently and therefore, there is immense potential in this fungus to be used as an entomopathogenic biocontrol agent for tea cultivation.

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Thrips (Insecta: Thysanoptera) fauna of Kaziranga National Park, Assam

Thrips are one of the economically important insects, belonging to the order Thysanoptera, having fringes on the wings with the body size ranging from 0.5 to 16 mm (ref. 1). The small body size of thrips is compensated by their appreciable breeding potential and quick development with at least 10–12 generations per year, especially under the tropical climate[2]. They display a great diversity in terms of feeding habit and habitat. With their piercing and sucking mouth parts, thrips feed on pollen grains, plant cell sap, fungal spore and mycelia, and even soft-bodied insects. Such feeding habits enable them to choose microhabitats like flowers, leaf sheaths, underneath barks, plant galls, leaf litters, etc. By virtue of their association with plants, some species could attain the status of primary pests, gall makers and vectors of plant diseases, besides pollinators[3–5]. The world record indicates occurrence of about 5864 species of thrips[6], of which nearly 700 species are known from the Indian subcontinent[7] and 205 from NE India[8]. However, only 16 species have been collected so far from Assam, including a new species *Neodixothrips assamensis* Sen & Muraleedharan[9]. The present report highlights thrips collections exclusively from the biodiversity-rich Kaziranga National Park (KNP), Assam.

KNP is one of the oldest national parks of Assam and it is a UNESCO World Heritage site, known for the Great Indian one-horned Rhinoceros. It covers an area of approximately 430 sq. km along the mighty Brahmaputra river in the north and the Karbi Anglong hills in the South, and is situated between 26°30′–26°45′N lat. and 93°08′–93°36′E long. (ref. 10). It is one of the largest tracts of protected land in the sub-Himalayan belt. The park is located in the Indo-Malaya ecozone; the dominant biomes of the region include Brahmaputra valley semi-evergreen forests of the tropical and subtropical moist broadleaf forest biome and a frequently flooded variant of the Terai–Duar savanna and grasslands of the tropical and subtropical grasslands, savannas, and shrub land biome. The park experiences summer, monsoon and winter seasons. The dry and windy summer extends approximately from February to May with the mean maximum and minimum temperatures of 37°C and 7°C respectively. The hot and humid monsoon persists from June to October. During the southwest monsoon, the park receives an average rainfall of 2220 mm/annum. The winter, extending from November to February, is mild and dry, with the mean maximum and minimum of 25°C and 5°C respectively[10,11]. The climatic conditions and varied vegetation make the park conducive for thrips to survive. They are abundant during March, April, September and October, which in turn, has enabled thrips collection during the above months. Specimens were sampled at random from grasses, plant gall, leaf litter and foliages of different plants following the standard techniques[3]. The collected specimens were balsam-mounted by conventional methods[8] and subsequently identified with standard keys available for the Indian fauna[12–15]. The permanent slides of the specimens are deposited in the PUSA collections, Indian Agricultural Research Institute, New Delhi. Prior permission was obtained from the Principal Chief Conservator of Forest/ Wildlife, Assam to collect the specimens