SUMMARY AND CONCLUSION
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A total of 152 soil samples were collected from 13 different habitats and majority of them were from paddy fields and forest lands. From these, 350 fungi and 94 actinomycetes were obtained. Their distribution in different habitats varied quantitatively. Most of the fungi were obtained from forest lands, paddy fields, canals, ponds and lakes and actinomycetes from forest lands, paddy fields and farmyard pits.

The fungi included representatives of 26 genera. Members of the genus Aspergillus accounted for the largest number and this was followed by Penicillium, Fusarium and Paecilomyces. The actinomycetes included members of 16 genera. The genus Streptomyces accounted for the largest number. This was followed by Micromonospora, Nocardia, Actinomadura, Actinoplanes and Nocardiopsis.

Extracellular and intracellular secondary metabolites obtained from all these fungi and actinomycetes were screened for mosquito larvicidal, ovicidal and adulticidal activity against Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti. Extracellular metabolites from 133 fungi and 35 actinomycetes were larvicidal, 34 fungi and 3 actinomycetes were ovicidal and 7 fungi
were adulticidal. Intracellular metabolites from 85 fungi and 9 actinomycetes were larvicidal, 18 fungi and 2 actinomycetes were ovicidal and 3 fungi were adulticidal.

The fungi which produced mosquitocidal metabolites included representatives of 21 genera. Most of them were *Aspergillus*, *Penicillium*, *Fusarium* and *Paecilomyces*. Members of the genera *Heterosporium*, *Sporotrichum*, *Geotrichum*, *Sporothrix*, *Thielavia*, *Trichosporium* and *Cladosporium* have been observed for the first time to produce insecticidal metabolites. While the metabolites produced by *Alternaria*, *Botrytis*, *Chaetomium*, *Gliocladium*, *Trichothecium*, *Trichoderma* and *Cephalosporium* have been recorded for the first time to produce mosquitocidal metabolites.

In actinomycetes, the mosquitocidal metabolites were obtained from 13 genera, of which *Streptomyces*, *Micromonospora*, *Actinoplanes*, *Nocardia* and *Actinomadura* were predominant. Members of the genera *Nocardia*, *Amorphosporangium*, *Dactylosporangium*, *Ampullariella*, *Elytrosporangium*, *Microbispora* and *Planomonospora* have been observed for the first time to produce mosquitocidal/insecticidal metabolites.

The mosquito larvae were more susceptible to both extracellular and intracellular metabolites of fungi and actinomycetes than eggs and adults.
Extracellular metabolites from 17 fungi and 5 actinomycetes were highly larvicidal. The fungi included 6 isolates of Aspergillus, 4 isolates each of Penicillium and Fusarium and one isolate each of Paecilomyces, Sporotrichum and Verticillium. The actinomycetes included 2 isolates each of Streptomyces and Actinoplanes and one isolate of Micromonospora. The LC50 values of the fungal metabolites were in the range of 3-24, 7-83 and 23-200 µl/ml against C. quinquefasciatus, A. stephensi and A. aegypti larvae, respectively. The LC50 of actinomycete metabolites were 1-17, 8-33 and 13-83 µl/ml against the respective larvae.

Intracellular metabolites from 8 fungi belonging to the genera Aspergillus, Fusarium and Penicillium and one actinomycete belonging to the genus Streptomyces were highly larvicidal. The LC50 values of fungal metabolites were in the range of 9-24, 14-41 and 21-96 µg/ml against C. quinquefasciatus, A. stephensi and A. aegypti larvae, respectively. The LC50 values of Streptomyces metabolite were 8, 12 and 22 µg/ml against the respective larvae.

Extracellular metabolites produced by 3 isolates of Aspergillus and one isolate each of Penicillium and Cladosporium and intracellular metabolites from 2 isolates of Aspergillus and one isolate of Fusarium caused egg mortality to appreciable level. Adulticidal activity was found only with fungal metabolites but at higher concentrations.
Extracellular metabolites produced by 2 isolates of *Streptomyces* (A81 and A34) and one isolate of *Paecilomyces* (F246) were highly toxic compared to others and the Lc50 values were respectively, in the range of 1-5 and 3 µl/ml against *C. quinquefasciatus*, 8-14 and 7 µl/ml against *A. stephensi* and 13-20 and 23 µl/ml against *A. aegypti* larvae.

Intracellular metabolites from 2 isolates of *Aspergillus* (F138 and F21) and one isolate of *Streptomyces* (A71) were highly toxic compared to others and the Lc50 values were respectively, in the range of 9-10 and 8 µg/ml against *C. quinquefasciatus*, 14-18 and 12 µg/ml against *A. stephensi* and 21-36 and 22 µg/ml against *A. aegypti* larvae.

The most promising fungi and actinomycetes were mass produced, their active compounds were isolated, purified and chemically and/or biologically characterized.

The larvicidal compound produced by the *Paecilomyces* isolate (F246) was identified as patulin. The compound produced by the *Aspergillus* isolate F21 was rubratoxin B. While the other isolate of *Aspergillus*, F138, produced 2 larvicidal compounds, one was aflatoxin B1 and the other G1.
The larvicidal compounds produced by the streptomycete isolates (A81, A34 and A71) were found different from each other and the compound produced by the isolate A81 showed the highest activity against the mosquito larvae.

The purified mosquitocidal compound from the *Streptomyces* isolate A81 was a colourless amorphous powder and soluble in water and organic solvents. It was heat stable and UV resistant and identified as an aromatic saturated ether ester. The compound was found novel and most active against the mosquito larvae, having LC50 values in the range of 0.4-2.7 ppm. It was found active against the housefly and cockroach. The compound also showed appreciable antifungal activity.

The macro- and micromorphological, cultural, physiological and biochemical characteristics of the producer organism demonstrated that it is a new strain of *Streptomyces griseus*.

When this streptomycete was grown in different media, the one containing glucose as carbon source and soybean meal as nitrogen source yielded higher amount of biomass and mosquitocidal compound. The growth pattern of the organism and the production of the mosquitocidal compound were studied and found that it reached the stationary phase of the growth on the 3rd of incubation and the production of the mosquitocidal compound started towards the end of
the exponential phase, i.e., after two days of incubation and was maximum on the 5th day of incubation. The optimal initial pH of the medium for the growth of this organism was 7.1 which was also optimum for the production of the mosquitocidal metabolite. The highest growth and highest amount of the mosquitocidal metabolite were obtained when the cultivation temperature was 28 -30°C.

The mosquitocidal metabolite of this new strain of *S.griseus* in both pure and crude forms exhibited neither lethal nor sublethal effect on non-target organisms including mammals.

The metabolite was equally effective in killing the mosquito larvae when tested in paddy field and casuarina pit waters whereas it was little less effective in highly polluted cess pit water.

In conclusion, the present study has identified a wide variety of fungi and actinomycetes that produce various kinds of mosquitocidal metabolites. Many of them have been observed for the first time not only to produce the mosquitocidal metabolites but to produce the insecticidal metabolites.

The intensive search has yielded a new strain of *Streptomyces griseus* that produces a novel mosquitocidal antibiotic. The compound
shows high potency against the mosquitoes. It also shows appreciable level of activity against the housefly and cockroach. It shows no toxicity in both mammalian and non-mammalian non-target organisms. The physical properties of the compound such as heat stability and UV resistance are desirable in the context of its field application. It effectively controls the mosquito larvae in non-polluted and less polluted environments. Thus, the compound is promising as a natural insecticide, because of its potent activity without toxicity to non-target organisms and animals.

As the producer organism utilizes inexpensive substrates, the production of this compound is economical. Further, as the compound is highly safe in the crude form itself, the culture broth of the organism can be directly used in insecticidal preparations. This eliminates the expensive chemical extraction and purification. And this possibility further merits the use of this compound.

As the compound exhibits appreciable antifungal activity and apparent mammalian safety, it can be also used as an antibiotic in medicine and agriculture.