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Short Communication

Bioefficacy of Aqueous Bulb Extract of Garlic (*Allium sativum* L.) and *Bacillus amyloliquefaciens* against Seed Borne Fungi of Okra

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The present investigation was undertaken to study the bioefficacy of aqueous bulb extract (w/v) of Garlic (*Allium sativum* L.) and *Bacillus amyloliquefaciens* against seed borne mycoflora of okra (*Abelmoschus esculentus* (L.) Moench) cultivated in Malabar, Kerala, India. Both local and hybrid (Salkeerthi) varieties were used in the study. *Abelmoschus esculentus* commonly referred to as lady's finger is an annual herb primarily valued for its edible pods. It is grown as a garden crop plant through out the tropical and sub-tropical parts of the world. The pods are rich in pectin and mucilage. A mucilaginous preparation from the pod has found application as a plasma replacement or blood volume expander [1]. The plant faces serious menace of fungal pathogen causing heavy losses in different agricultural conditions. Moreover, repeated use of fungicides may lead to the problems like environmental pollutions and the pathogen developing resistance to the fungicides.

Control of seed borne pathogenic and saprophytic fungi is possible through a biocontrol agent. Therefore, developing biocontrol measures becomes imperative. So it is felt necessary to find out an alternative measure for developing an effective broad based, cheap, eco-friendly method which is easily accessible to farmers.

Seven samples each of local and hybrid seeds (Salkeerthi) were collected from different locations of Malabar, Kerala both during rainy and summer seasons.

Germination percentage and vigour index: The criterion applied to differentiate germinated seeds from ungerminated seeds were a well developed root system including a primary root, and a well developed and intact hypocotyls without damage to the conducting tissues and normal plumule and two cotyledons for seedlings [2]. The root and shoot length were measured on the 11thday to calculate the seed vigour.

Vigour Index (VI) = [Average shoot length + Average root length] x Germination percentage [3].

Isolation and identification of pathogens: Identification of seed borne mycoflora were carried out by standard blotter method and pathogenicity of dominant isolated fungi was conducted *in vitro*. Fungal flora (*Aspergillus flavus, A. niger, Fusarium moniliforme* and *Macrophomina phaseolina*) were isolated from diseased okra seedling on PDA. The cultures of pathogens were purified and then their identity was confirmed. Subsequently purified cultures were multiplied and maintained in Petri plates.

Garlic extract was prepared by crushing 500g of cleaned garlic cloves in 500 ml water. Non crushed fibrous tissues were filtered through muslin cloth. The resultant was considered 100 per cent concentration.

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Screening of antagonist: Bacillus amyloliquefaciens was isolated from soil samples collected from okra gardens by serial dilution method to get 1 x 10 colony forming unit / ml on nutrient agar. The bacterial culture was purified by single spore isolation and the identity was confirmed. Subsequently purified culture was multiplied and maintained in Petri plates. Further bacterial formulations were prepared by mixing 100ml bacterial suspension with 250g of commercially available sterilized and purified talcum powder under sterile condition. Seeds were treated with freshly prepared *Bacillus amyloliquefaciens* at the rate of 0.5g/50g of seeds for 24 hours.

The antagonism between the aqueous extract of *Allium sativum* and *Bacillus amyloliquefaciens* and pathogenic fungi isolated from *Abelmoschus esculentus* seeds was studied by adding the extract of *Allium sativum* to the PDA medium and sterilizing it. Twenty ml. of sterilized and melted PDA was poured into the sterilized Petri plates (90mm). After solidification, the plates were inoculated with test fungus using 5mm diameter discs of actively growing colony. In another Petri plate with PDA medium 5mm diameter disc of test fungus was inoculated at one side and streak of *Bacillus amyloliquefaciens* was made at the opposite end of the Petri plate. The plates were incubated at a 25±2°C for ten days and the observation was recorded. Each treatment was replicated thrice. At the end of the incubation period, radial growth of the pathogen and the test bacteria was measured both towards the interaction side and side of the Petri plate. Percentage inhibition of fungi was determined by applying the formula of Skidmore [4]

$$\frac{C\text{-}C1}{C} \times 100$$

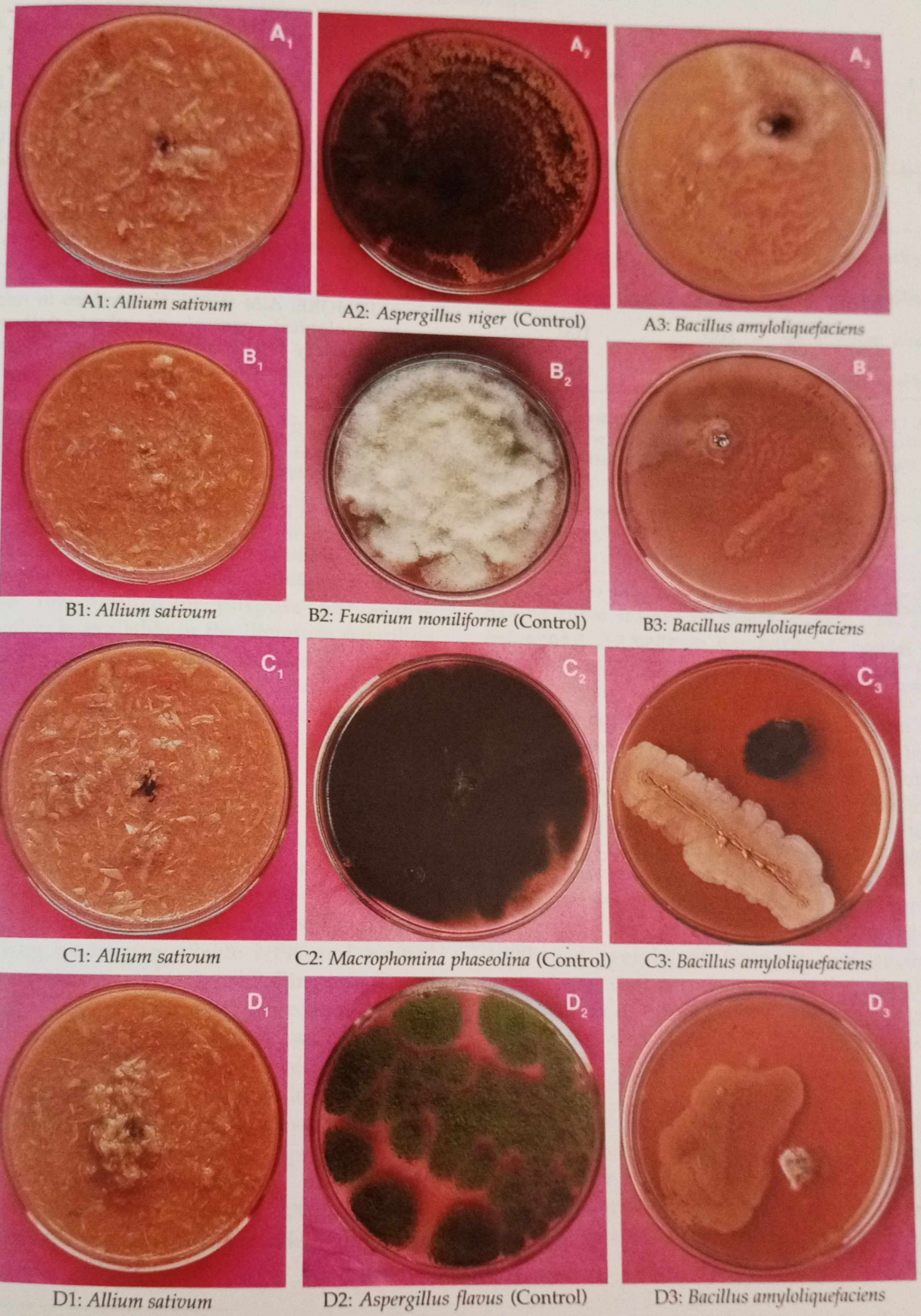
where C represents the distance in mm of fungal growth from the point of inoculation to the colony margin and C1 is the growth of fungus towards antagonist.

In vitro studies of aqueous bulb extract of garlic showed promising antifungal activity having 100 per cent inhibition of *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* and *Macrophomina phaseolina*, compared to control having 100 per cent mycelial growth. (Fig. 1. and Table 1). Bulb extract treated seeds *in vivo* showed high germination (99%) and vigour index (Table 2) and complete absence of *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* and *Macrophomina phaseolina*. Purohit and Vyas [5] reported that the essential oil obtained from the bulbs of *Allium sativum* contains diallyl disulphide and other sulphur compounds. The active principle is allicin and a number of antibiotic principles like allistatin, allistatin 2 and garlic pulp is regarded as important for chronic colitis, angina pectoris, bacterial and fungal infections, amoebiasis, arteriosclerosis, rheumatoid arthritis and cancer.

Similarly *in vitro* study using *Bacillus amyloliquefaciens* also showed complete inhibition of *Fusarium moniliforme (100%)* followed by *Aspergillus flavus (83%)* and *Aspergillus niger and Macrophomina phaseolina* (75%) respectively, compared to control having 100 per cent mycelial growth (Fig. 1. and Table 1). Gupta [6] reported that *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme* were internally seed borne and produce varying degree of seed and seedling mortalities.

In vivo studies of garlic extract: 400 seeds were soaked in aqueous extract at a concentration of 1:1 (w/v). Seeds were soaked in aqueous extract for eighteen hours. The treated seeds were air dried at room temp, for a period of 10-12 hours and 100 seeds were placed on moist blotting sheet measuring $30cm^2$ which were rolled and incubated for 11 days. On the 11^{th} day, germination percentage of seeds and root-shoot length of the seedlings were measured. Garlic extract and *Bacillus amyloliquefaciens* treated seeds showed high levels of seedling emergence and low levels of pre emergence mortality compared to control. Germination efficiency of garlic was 99 per cent and *Bacillus amyloliquefaciens* was 83.5 per cent and showed high vigour index (Table 2).

Present experiments undoubtedly proved that *Bacillus amyloliquefaciens* and aqueous bulb extract of garlic were most effective for controlling seed borne pathogenic and saprophytic fungi of local and hybrid (Salkeerthi) of okra cultivated in Malabar, Kerala, India. Compared to *Bacillus amyloliquefaciens*, aqueous bulb extracts of garlic



A1: *Allium sativum* A2: *Aspergillus niger* (Control) A3: *Bacillus amyloliquefaciens*

B1: *Allium sativum* B2: *Fusarium moniliforme* (Control) B3: *Bacillus amyloliquefaciens*

C1: *Allium sativum* C2: *Macrophomina phaseolina* (Control) C3: *Bacillus amyloliquefaciens*

D1: *Allium sativum* D2: *Aspergillus flavus* (Control) D3: *Bacillus amyloliquefaciens*

Fig. 1. A1-A3: Effect of *Allium sativum* and *Bacillus amyloliquefaciens* on the growth of *Aspergillus niger* under *in vitro* condition; B1-B3: Effect of *Allium sativum* and *Bacillus amyloliquefaciens* on the growth of *Fusarium moniliforme* under *in vitro* condition; C1-C3: Effect of *Allium sativum* and *Bacillus amyloliquefaciens* on the growth of *Macrophomina phaseolina* under *in vitro* condition and D1-D3: Effect of *Allium sativum* and *Bacillus amyloliquefaciens* on the growth of *Aspergillus flavus* under *in vitro* condition

Table 1. ***In vitro*** **inhibition (%) of dominant pathogenic fungi isolated from *Abelmoschus esculentus* by *Bacillus amyloliquefaciens* and *Allium sativum* bulb extract**

Fungi	*Bacillus amyloliquefaciens*	*Allium sativum*
Aspergillus flavus	83.3	100
Aspergillus niger	75.0	100
Fusarium moniliforme	100	100
Macrophomina phaseolina	75.0	100

Table 2. Per cent germination and vigour index of *Abelmoschus esculentus* seed treated with *Bacillus amyloliquefaciens* and *Allium sativum* bulb extract under *in vivo* conditions

	Bacillus amyloliquefaciens	*Allium sativum*	Control
Germination (%)	83.5	99	77
Vigour index	1208	1257.5	1024

Data based on 400 seeds

proved more efficient as a bio control agent, hence the need for developing an effective broad based, integrated control of seed borne pathogens and decay causing saprophytes by eco-friendly methods were the need of the new millennium because seed is the basic unit of crop production technology.

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