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Research Article

Effectiveness of Selected Homeopathic Medicines Against *Colletotrichum gloeosporioides*

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Abstract

Background and Objective: Anthracnose caused by *Colletotrichum gloeosporioides* (Penz. & Sacc.) is one of the most important diseases of *Mangifera indica* L. Recent studies have focused on the development of environmentally safe, long-lasting and effective bio-control methods for the management of anthracnose. The objective of this study was to design appropriate control measure against *C. gloeosporioides* by using selected homeopathic medicines of different potencies and concentrations.

Materials and Methods: Seventy two homeopathic medicines each with 5 Centesimal Hahnemannian potencies containing anti-fungal properties were selected. Food poisoning method was adopted and data were collected on percentage of inhibition. The experiment was laid out in Completely Randomized Design (CRD) with 5 replications. **Results:** *Arsenicum album* (64.62%) and Q potency (16.52%) was significantly ($p < 0.0001$) effective against *C. gloeosporioides* followed by the order of *Arsenicum album* < *Selenium* < *Nuxvomica* < *Belladonna* < *Calcarea fluorica* and Q < 3X < 200X < 30X < 1 M, respectively and the rest were below. A checking of 9 potencies of *Arsenicum album* Q potency significantly inhibited (82.93%) at 3000 ppm individually and followed the order of Q < 3X < 6X < 30X < 50 M < 1 M < 200 M < CM < 10 M. Among the 7 concentrations, 10000 ppm of *Arsenicum album* Q potency significantly inhibited (96.40%) at 10000 ppm concentration. Treating with *A. album* Q at 10000 ppm on the 3 varieties of mango, himsagar (18.3%) showed the significant inhibition of percent disease incidence over control and followed the order of Himsagar < Amrapali < Langra. **Conclusion:** *Arsenicum album* Q (at 10000 ppm) significantly inhibited (96.40%) the growth of *C. gloeosporioides*.

Key words: *Colletotrichum gloeosporioides*, inhibition, homeopathic medicines, potencies, concentrations

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colletotrichum gloeosporioides (Penz. & Sacc.) is the causal agent of anthracnose of *Mangifera indica* L. (mango). The disease is very harmful and can cause spoilage and rotting of fruits, resulting in low yield and poor quality of the fruits¹. The utilization of chemical fungicides is frequently picking for controlling of anthracnose disease, but this also causes the expansion of fungal resistance². However, continuous and inappropriate use of chemical fungicide to control anthracnose disease is not well thought-out to be the durable solution because this can boost the investment, the threat of having high levels of poisonous residues and also the concerns in human health and environmental set-up³. As a result, several attempts were made to search for alternative measures to control the anthracnose disease effectively. The works have focused on the advance of environmentally secure, enduring and efficient bio-control methods for management of anthracnose diseases. The exploitation of natural products, particularly the homeopathic medicines, were shown to be effective against plant pathogens and considered to be safe for consumers and environments³. A number of homeopathic medicines have been reported to possess antifungal substances that are poisonous to a range of plant pathogenic fungi and highly diluted and successes⁴. Toledo *et al.*⁵, achieved significant outcome in the control *Alternaria alternata*, *C. gloeosporioides*, *Fusarium roseum* and *Gloeosporium psidii* causing mango, guava and tomato fruit rots by the use of homeopathic medicines in post-harvest period. Dahiwale and Suryawanshi⁶ observed that certain homeopathic medicines were inhibitory against *Alternaria alternate* and *Botrytis cinerea* causal agent of fruit rot of pomegranate, grey mould of grape, respectively.

Homeopathic medicines were discovered to be useful among different plant pathogenic fungi in a number of cost-effective crops. *Botryodiplodia* in guava fruit, control of *Aspergillus niger* in coriander and cumin, betel vine disease caused by *Phytophthora parasitica* var. *piperina* and *Aspergillus niger* in sisal plants⁷, *Alternaria brassicicola* in dark leaf spot of cauliflower⁸, blight of cotton caused by *Xanthomonas campestris* pv. *malvacearum*⁹ and *Arabidopsis thaliana* infected with *Pseudomonas syringae*¹⁰. In recent years, homeopathic medicines have been used *Lycopodium* 140 for *Pestalotia mangiferae*, *Kali iodatum* 200 for stem-end rot and *Rhizopus* rot of mango fruits and *Methyl jasmonate* 200 for *Fusarium* fruit rot of banana for the control of post-harvest losses¹¹. Wyss *et al.*¹² suggested that homeopathic therapy selection, potency level and dosages for further optimization. In this sense, homeopathic drugs might

be used in a possible way to control the incidence of plant diseases. It also helps people for fresh fruit consumption because of their easy availability and use, cheap and no side effect. However, this study will contribute new science in advance that antifungal effect of explicit homeopathic medicines with their potency and concentration against *C. gloeosporioides* to reduce yield loss as well as post-harvest loss and increase shelf life of mango under laboratory.

Presently, this science is being used in different branches of agriculture, mainly in the area of plant protection⁵. The homeopathy can support in the safeguarding of plants health¹³ for its stimulating effect and/or inhibiting effect directly on the pathogens. Moreover, homeopathy is inexpensive substitute to chemical fungicides. Therefore, this experiment was conducted to explore the appropriate control measure against *C. gloeosporioides* by applying selected homeopathic medicines of different potencies and concentrations.

MATERIALS AND METHODS

Collection, isolation and pathogenicity test of *C. gloeosporioides*. *Colletotrichum gloeosporioides* isolates were collected from anthracnose lesions of mango fruits (*Mangifera indica* L.) from Satkhira (January 2015) of Bangladesh. Samples (5 × 5 mm) were taken from healthy and margin of diseased tissue. Samples were sterilized by dipping in 1% sodium hypochlorite for 2 min and washing in 3 times through sterile water. Plates were incubated at 25 ± 2°C (room temperature) and observed periodically. The growing edges of fungal mycelium were transferred aseptically to Oat Meal Agar (OMA) containing Petri plates. The isolates were recognized based on morphological characters. The presence of unicellular, hyaline and oval shape conidia will be *C. gloeosporioides*. The fungi were stored at 4°C on OMA slants.

The identified isolates of *C. gloeosporioides* were confirmed by Koch's Postulates methods/Tissue planting method on Oat Meal Agar (OMA) containing Petri plates. Healthy mango fruits were placed in petri-plates with 3 replications under laboratory conditions. The conidial suspension 5 × 10⁶ spore mL⁻¹ (supplemented with 0.1% Tween 20 1:1 w/v) was prepared by using haemocytometer. Symptom appeared after 5 days of inoculation. Small brown spot appear on fruits and the spots gradually enlarged, center of the lesions turned dark brown with numerous acervuli with masses of spores observed under stereo microscope. The glass slide was prepared and observed under compound microscope of 400 × magnifications. The presence of conidia is similar to those of original isolate (Fig. 1).

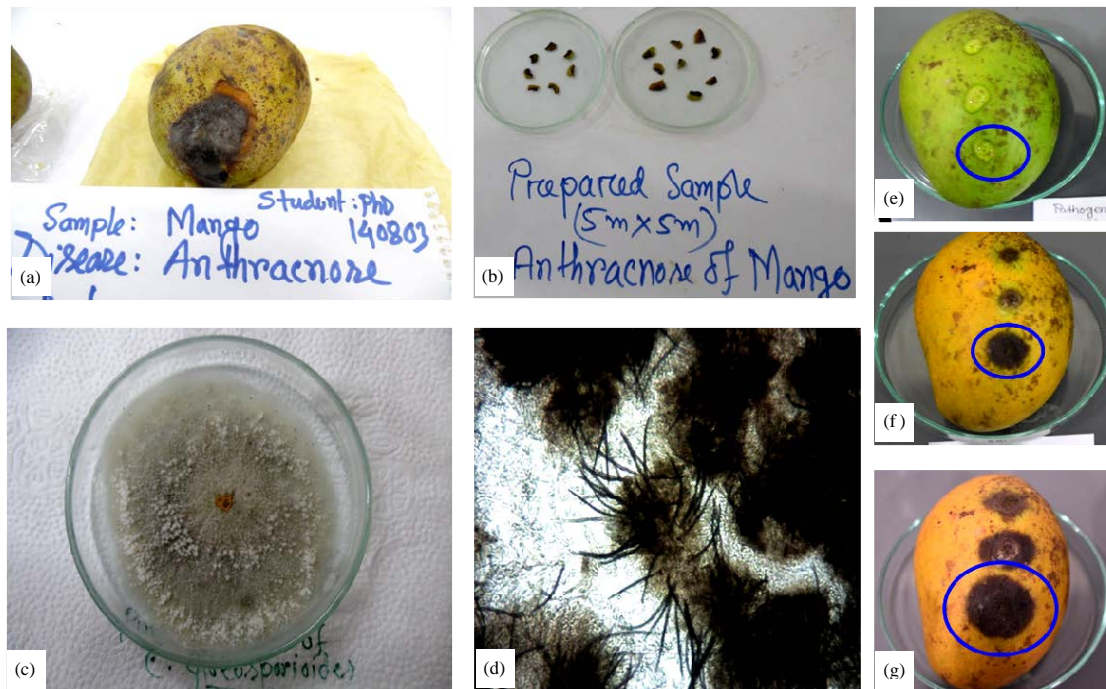


Fig. 1(a-g): Isolation and pathogenicity test of *C. gloeosporioides* from anthracnose affected mango on Oat Meal Agar (OMA) containing Petri-plates (a) Mango fruit, (b) 5 mm cut sample, (c) Pure culture, (d) Setae with conidia (400X), (e-g) Symptoms produced on healthy mango after 7 and 14 days

Selection of homeopathic medicines with their potencies:

Seventy two homeopathic medicines were selected based on anti-fungal properties considered by Gama *et al.*⁷ and it was purchased from wholesale market of Khulna city. Five Centesimal Hahnemannian potencies (Strength of a homeopathic remedy) viz. Q (Mother tincture), 3X (A potency of 3C means 1: Dilution of the original substance), 30X (A potency of 30C means 1: Dilution of the original substance), 200X (A potency of 200C means 1: Dilution of the original substance) and 1 M (A potency of 1 M means a dilution of 1 part in 1000) were randomly selected to screen their efficacy against *C. gloeosporioides*. A commercially available complex homeopathic preparation specially made for human was tested through food poisoning method and observing the percentage of inhibition against *C. gloeosporioides*.

Preparation of Oat Meal Agar (OMA) medium, mixing of medicine with OMA, pouring and solidification: Oat Meal Agar (OMA) medium was prepared following the standard procedure¹⁴. Prepared 20 mL OMA containing test tube and it was melted by oven. After melting, it was kept few minutes for reducing heat. Before solidifying, each potency of

homeopathic medicine was added 60 ppm/20 mL separately in each test tube by micropipette. Then it was mixed by shaking. For control treatment no medicine was added. Then treated test tubes were poured in each sterilized Petri dishes. All the activities were undertaken under aseptic condition (Laminar air flow hood). Each plate was considered as a replication. Five replications were used for each potency. The all potencies of 72 homeopathic drugs were separately added to make the concentration 3000 ppm medium in 90 cm Petri dishes.

Concentration of each medicine: Seven different concentrations were prepared such as 80, 100, 120, 140, 160, 180 and 200 ppm/20 mL OMA medium containing test tubes to make the concentrations 4000, 5000, 6000, 7000, 8000, 9000 and 10000 ppm, respectively. Mixing media were poured into Petri plates. Percentage Control Efficacy (PCE) was determined by food poisoning method².

Procedure of treating mango against *C. gloeosporioides*
Three varieties each of 5 healthy mangoes were collected from the orchard. Mangoes were treated with the selected homeopathic medicine immediately after collection.

Approximately 10000 ppm of *Arsenicum album* Q was added with 1 L distilled water by micropipette. Selected mangoes were dipped for 10 min. After treating, it was kept in chamber for observation.

Evaluated parameters

Percent control efficacy of *C. gloeosporioides*: The radial growth of hyphae was recorded after 8 days the 80% growth of mycelium in control plate (cross measurement was done at right angles). The antifungal homeopathic medicines were tested individually against mycelial growth of *C. gloeosporioides* using Oat Meal Agar (OMA) medium by food poisoning method³. Percentage Control Efficacy (PCE) was determined using Eq. 1³:

$$\text{Percent Control Efficacy (PCE)} = \left(\frac{C-T}{C} \right) \times 100 \quad (1)$$

Where, C is control and T is treated.

Disease incidence: Disease incidence was assessed after 18 days of incubation with and without symptoms of anthracnose of mangoes.

The disease incidence on fruits was assessed using a 0-5 scale. Where, 0 = No infection, 1 = Up to 5%, 2 = 6-10%, 3 = 11-20%, 4 = 21-50%, 5 = More than 50%. Based on the 'Percent disease index' for anthracnose of mango was calculated using the Eq. 2¹⁵:

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of infection ratings}}{\text{No. of fruits examined} \times \text{Maximum grade}} \times 100 \quad (2)$$

Latent period, lesion size, infection frequency, lesion cover and acervuli production on *Mangifera indica* L.:

To evaluate Latent Period (LP) in days (first symptom appears) data was collected from the 3 variety of mangoes. Lesion Size (LS) was measured in cm (Lesion size = Lesion diameter × length), infection frequency % (IF) = Number of spots/Lesion size × centroid factor (2/II or 0.63)¹⁶; Lesion Cover (LC) (cm) = Lesion size × Infection frequency and number of acervuli (A) per square cm data were collected.

Data analysis: The conducted experiment was Completely Randomized Design (CRD) with five replicates. The data were analyzed by Statistical Analysis System (SA) program version 2 and means were compared following New Duncan's Multiple Range Test (DMRT).

RESULTS

Effect of medicines their corresponding potencies and concentrations:

The result showing the fungi toxic effects of 72 homeopathic medicines with 5 potencies are presented in Fig. 2. It is evident from this results that all the homeo drugs showed remarkable efficacy for checking the *Colletotrichum gloeosporioides* mycelia growth development. The highly significant difference ($p < 0.0001$) of *C. gloeosporioides* was shown by *Arsenicum album* than *Selenium*, *Nux vomica*, *Belladonna* and *Calcarea fluorica* were found to be most efficacious amongst all the tested homeopathic medicines. *Selenium* was no significant ($p < 0.0001$) than *Nux vomica* and *Belladonna* was no significant than *Calcarea fluorica*. *Selenium* and *Nux vomica* was significant ($p < 0.0001$) than *Belladonna* and *Calcarea fluorica* Q potency was shown significantly highest efficacy (16.52%) than 3X (15.71%), 30X (13.57%), 200X (14.50%) and 1 M (12.10%). The 3X was no significant than 200X but significant from 30X and 1 M. There was no significant differences ($p < 0.0001$) among the replication of *Kali Bich*, *Hepersulph*, *Carbonica*, *Bryonia* and *Borax* (Table 1, Fig. 2).

Mycelia growth of *C. gloeosporioides* were considerably balanced by 9 available potencies (Q, 3X, 6X, 30X, 1 M, 10 M, 50 M, 200 M and CM) of *Arsenicum album* at 3000 ppm concentration. The Q potency induced significantly ($r^2 = 0.97$, $p < 0.0001$) maximum growth inhibition and the lowest 10M potency. 3X, 6X and 30X were significant with themselves. Similarly lower inhibition of CM potency which gradually increased with increase in the concentration of homeo drugs viz. 200 M, 1 M, 50 M, 30X, 6X and 3X were not significantly different (Fig. 3a-b).

Seven concentrations of Q potency of *Arsenicum album* were used to determine for their efficacy against *C. gloeosporioides* mycelia growth on OMA media. About 10000 ppm concentration of *Arsenicum album* Q was

Table 1: Effectiveness of best 5 homeopathic medicines out of 72 against *C. gloeosporioides* on OMA containing Petri-plates

Homeopathic medicines	Percentage of inhibition (Mean)
<i>Arsenicum album</i> (Q)	64.62 ^a
<i>Selenium</i> (1 M)	57.76 ^b
<i>Nux vomica</i> (Q)	57.13 ^b
<i>Belladonna</i> (Q)	39.48 ^c
<i>Calcarea fluorica</i> (3X)	36.92 ^c
CV%	55.73
Pr > F	0.0001
LSD	4.47

OMA: Oat Meal Agar, ^{a-c}Values followed by different letters are not significantly different ($p < 0.0001$)

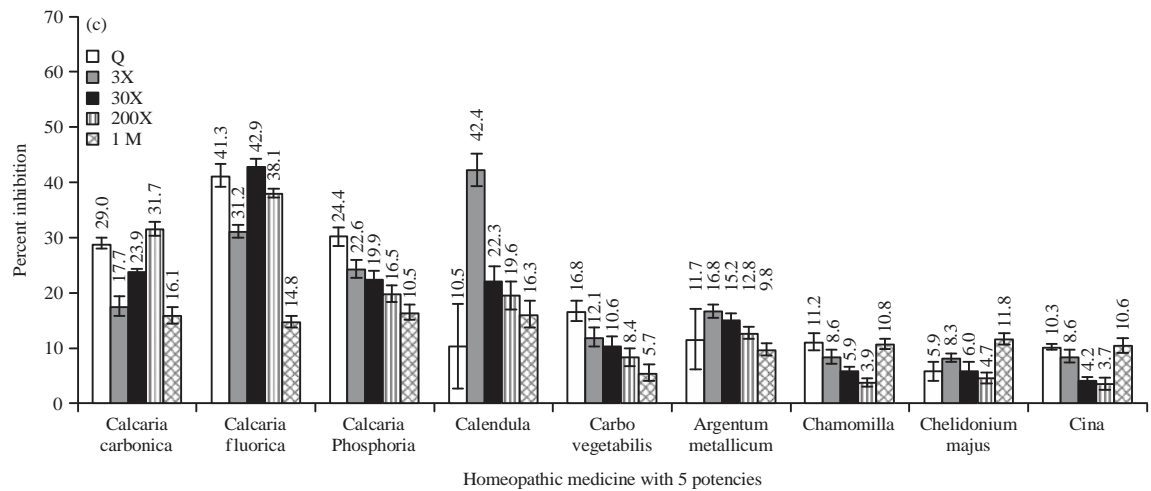
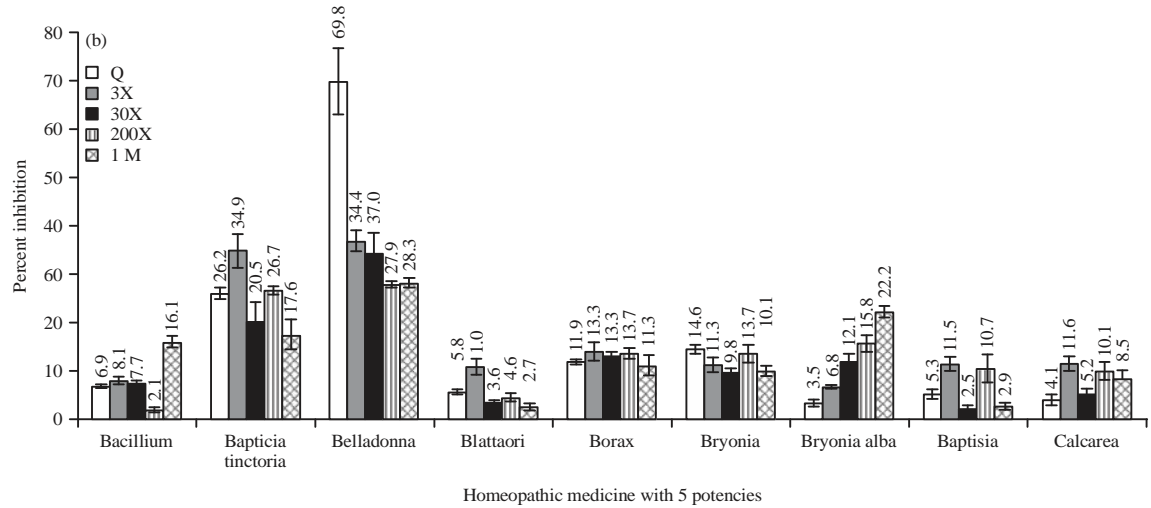
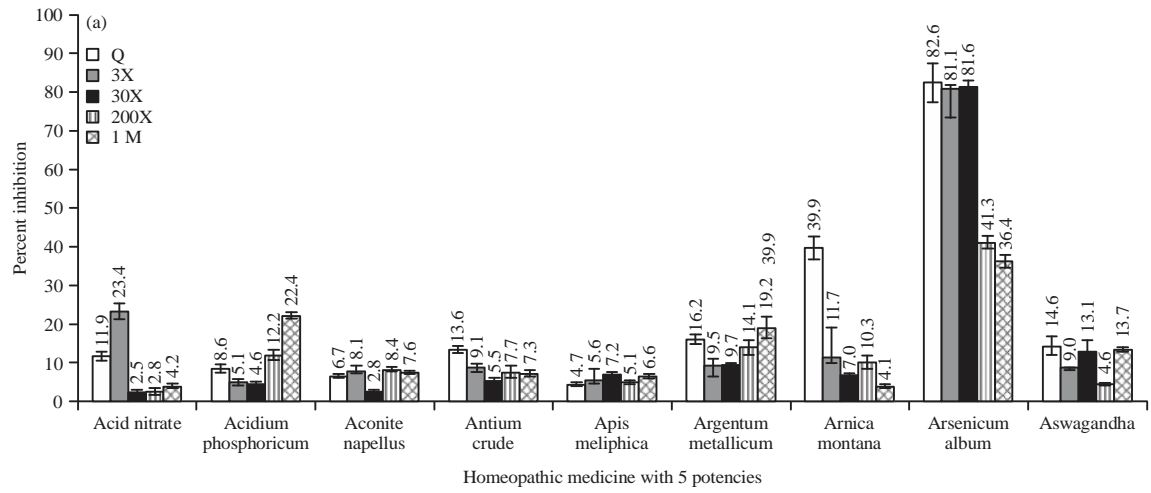


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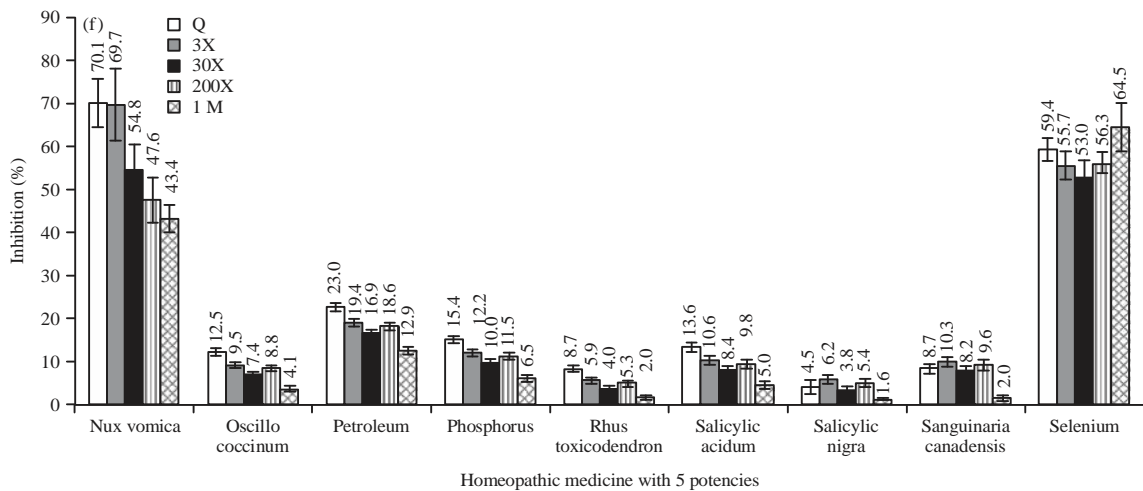
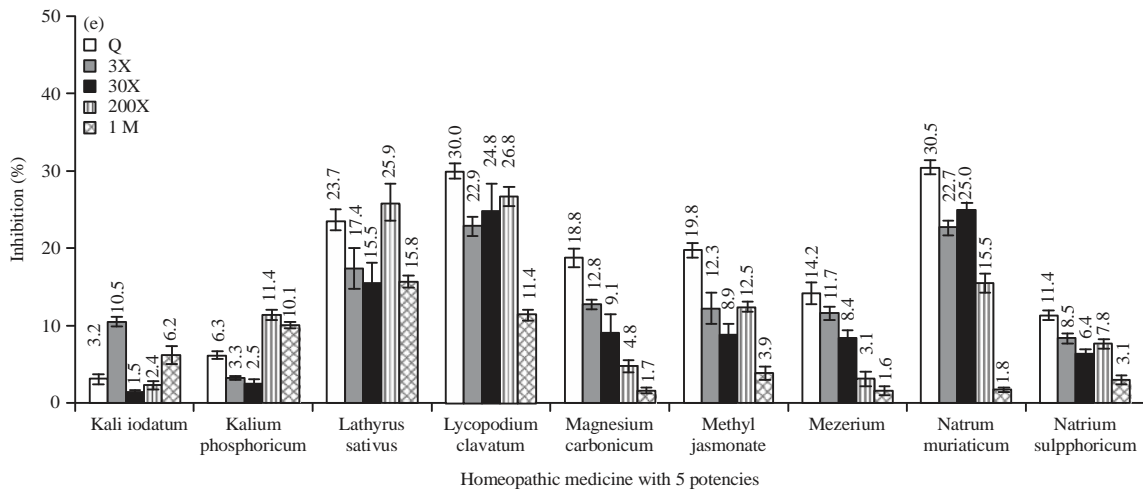
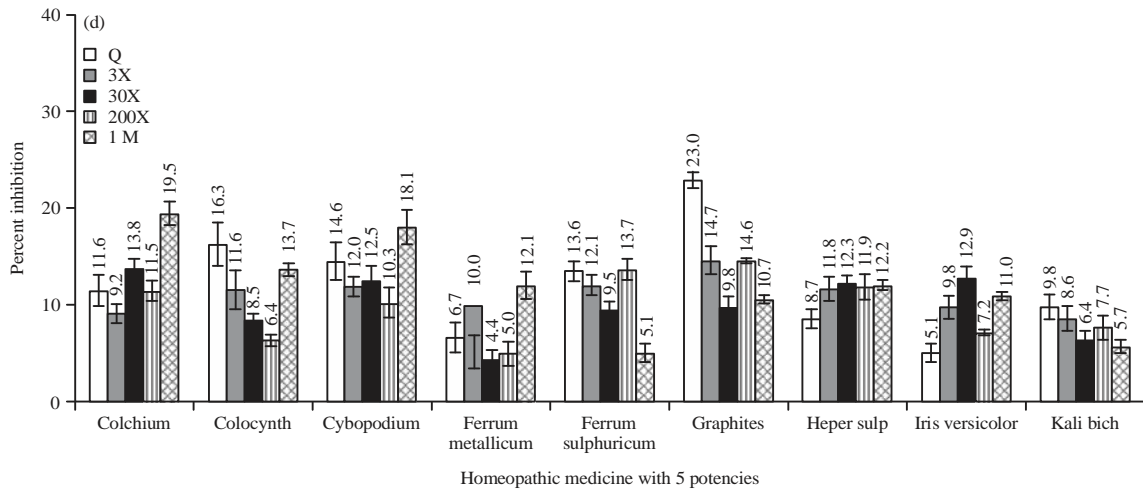


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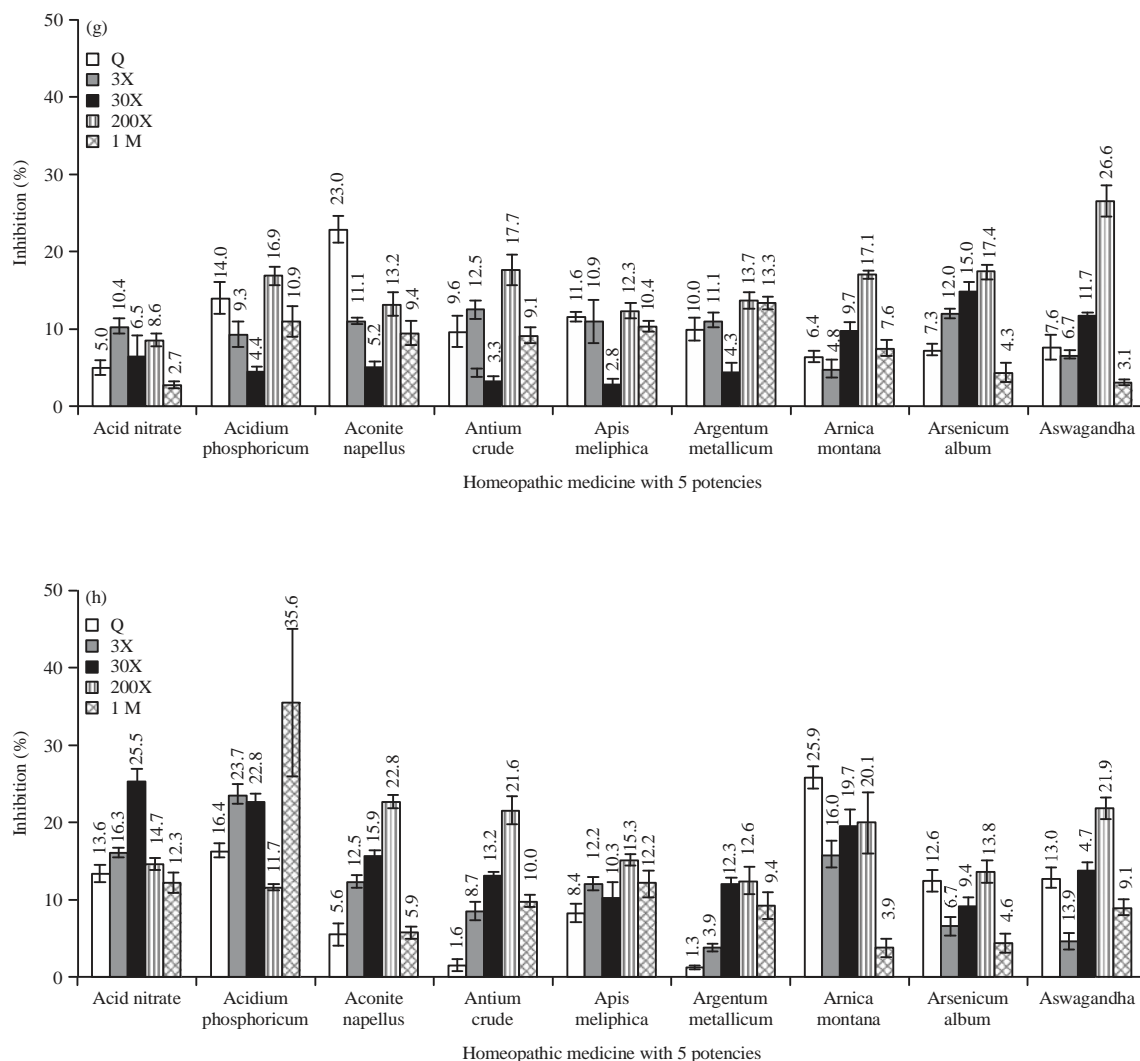


Fig. 2(a-h): Mean percent efficacies over control of 72 homeopathic medicines with their 5 potencies against *C. gloeosporioides* on Oat Meal Agar (OMA) containing Petri-plates

significantly ($r^2 = 0.95$, $p < 0.0001$) inhibited the maximum 96.40% growth of *C. gloeosporioides*. However, the other concentrations exhibited 9000, 8000, 7000 and 6000 ppm significant difference. About 5000 and 4000 ppm antifungal activity against *C. gloeosporioides* were not significantly different (Fig. 3c-d).

Effect of homeopathic medicine on mango: *Arsenicum album* Q at 10000 ppm concentration was used for the management of *C. gloeosporioides*. Eighteen days after treating himsagar (18.3%) was highly significant inhibition of Percent Disease Incidence (PDI) than amrapali (8.2%) and langra (0%) where 100% disease incidence of the control of three varieties. Lesion cover, lesion size and acervuli production were the lowest in mangoes as compare to control. Latent period and infection frequency were

highest 2.0 days and 1.2%, respectively than control. All the 3 varieties LS, LC and A were highest in langra than control (12.5, 25.4 and 2.0/square cm) and lowest in himsagar (4.3, 16.5 cm and 1.6/square cm). The LP and IF were highest on himsagar and lowest on langra (5.6 cm and 2.6%) than control (4.4 cm and 2.1%) (Fig. 4).

DISCUSSION

Results in this study inferred that all of the selected homeopathic medicines exhibited significant ($p < 0.0001$) antifungal effect against *C. gloeosporioides* at 3000 ppm on OMA contained Petri-plates. Among the tested medicines *Arsenicum album* showed significant ($p < 0.0001$) inhibition against *C. gloeosporioides* in controlling the diseases.

Homeopathic medicines are reported to be effective to control plant disease. Baviskar and Suryawanshi¹⁷ reported *Arsenicum album* have an inhibitory activity against fungi responsible for fruit rot diseases. Asha *et al.*¹⁸ reported that *Thuja occidentalis* had inhibitory effect against *Bipolaris* spp. Singh *et al.*¹⁹ has reported *Belladonna* was found most effective drug on growth and aflatoxin production of

Aspergillus flavus. Dahiwale and Suryawanshi⁶ by using homeopathic medicine reported that grey mould of grape is caused by *Botrytis cinerea*.

Market available 9 potencies of *Arsenicum album* Q were found to significantly ($p < 0.0001$) inhibit (82.93%) *C. gloeosporioides* growth at 3000 ppm. The study proved that *Arsenicum album* Q at 10000 ppm was found to be

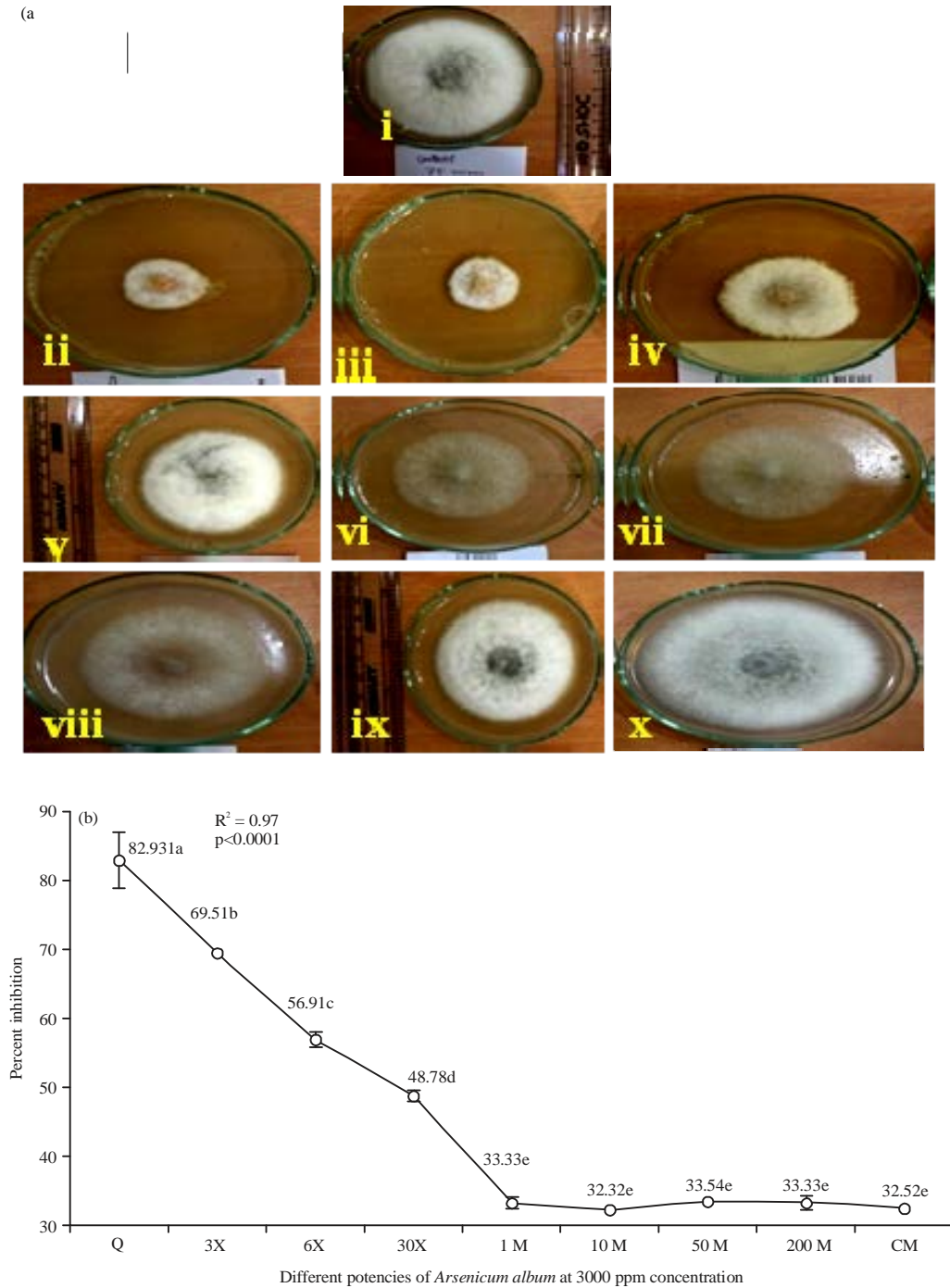


Fig. 3(a-d): Continue

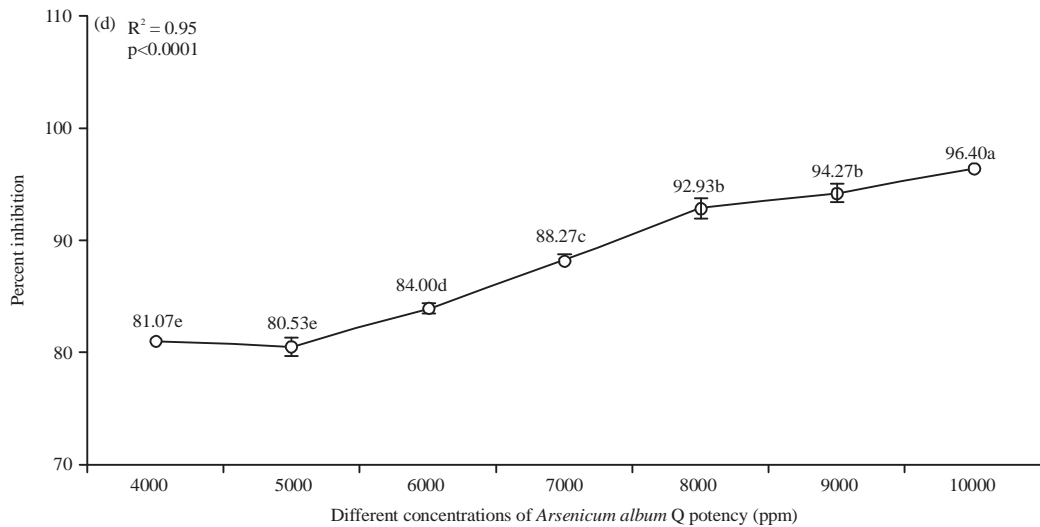
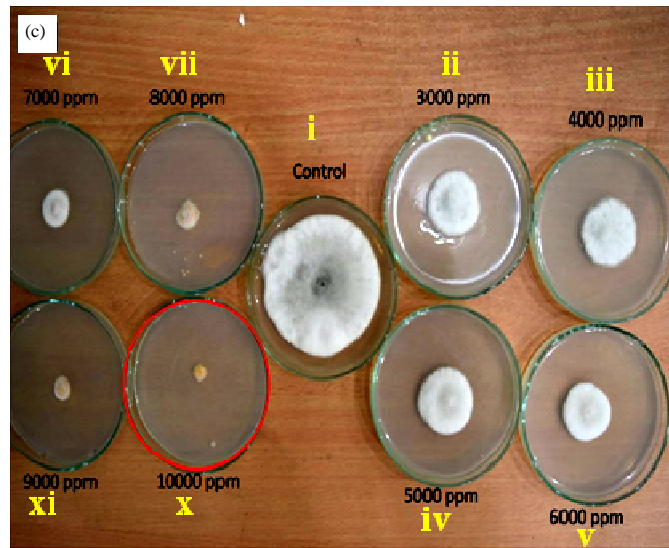


Fig.3(a-d): Mean percent efficacies over control of 9 potencies and 7 concentrations of *Arsenicum album* against *C. gloeosporioides* on OMA medium, (a-b) Nine potencies and graph showing the percent inhibition ($r^2 = 0.97$, $p < 0.0001$), (c-d) Seven concentrations and graph showing the percent inhibition ($r^2 = 0.95$, $p < 0.0001$)

significantly effective (96.40%) against *C. gloeosporioides*. Hanif and Dawar²⁰ and Hanif *et al.*²¹ reported that *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* significantly ($p < 0.05$) inhibited and also enhanced growth parameters and yield of crops by using *Arnica montana* and *Thuja occidentalis*. *Arsenicum album* 88% reduction was observed at 200 potency of leaf rot of betel vine *Phytophthora* disease.

The study discovered the potential of *C. gloeosporioides* through *Arsenicum album* Q at 10000 ppm concentration as a natural source of fungicidal material in controlling the

severity of anthracnose of mango. It would be helpful for the researchers that homeopathic medicines have the prospective to inhibit the growth of *C. gloeosporioides*. It can be beneficial that post harvest loss of mangoes were easily controlled by homeopathic treatment without any side effect. Thus, a new concept of homeopathic treatment against *C. gloeosporioides* with their potency and concentration and possibly to reduce post-harvest loss and increase self-life under laboratory. In further study, field application is necessary to prevent yield loss as well as post-harvest loss without any risk of health hazards.

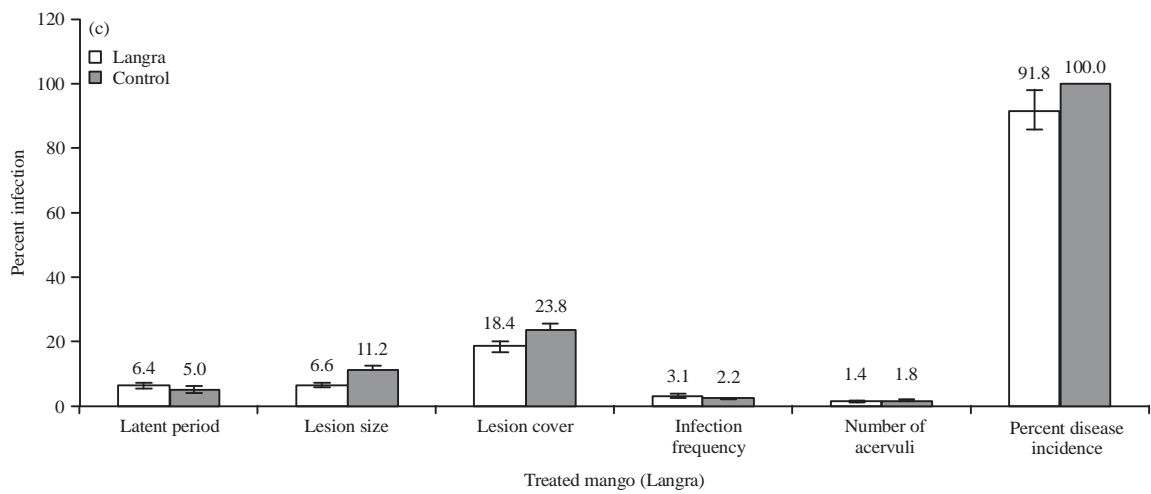
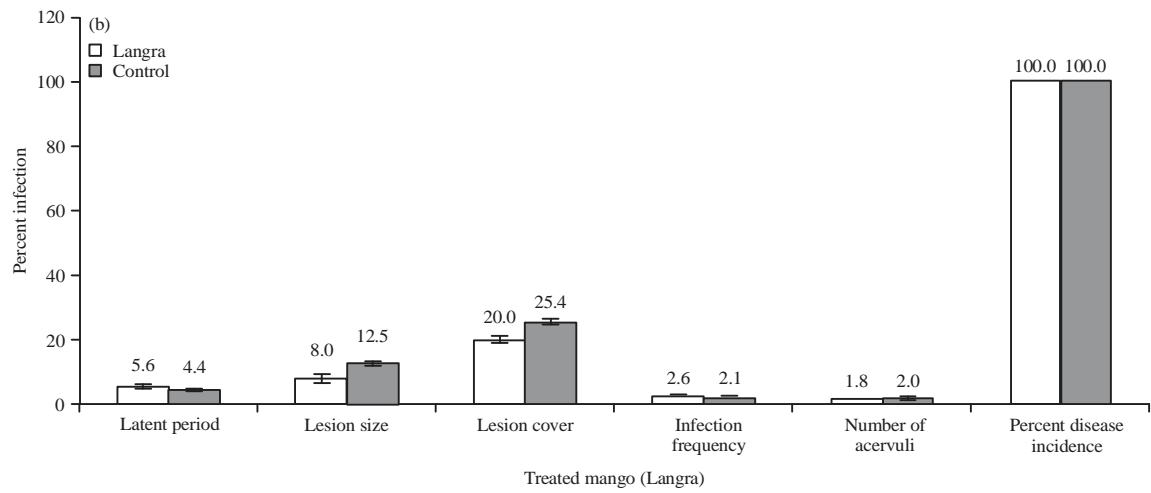
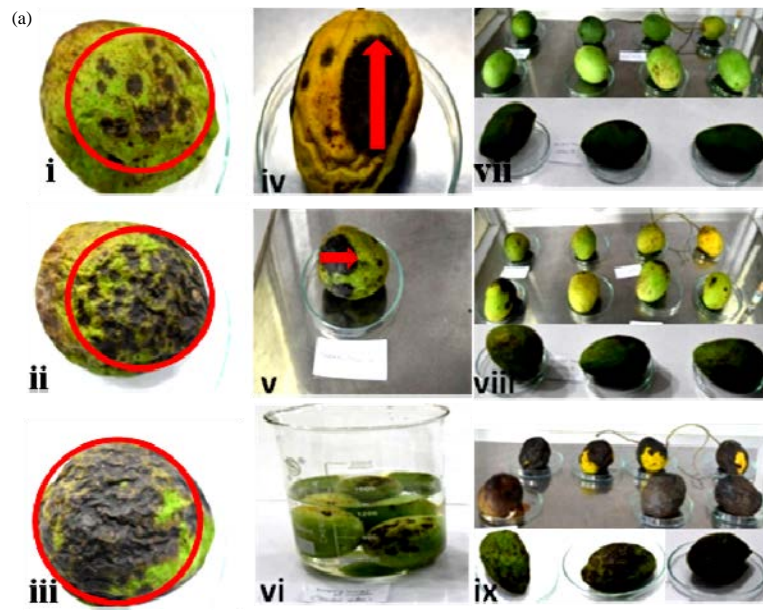


Fig. 4(a-d): Continue

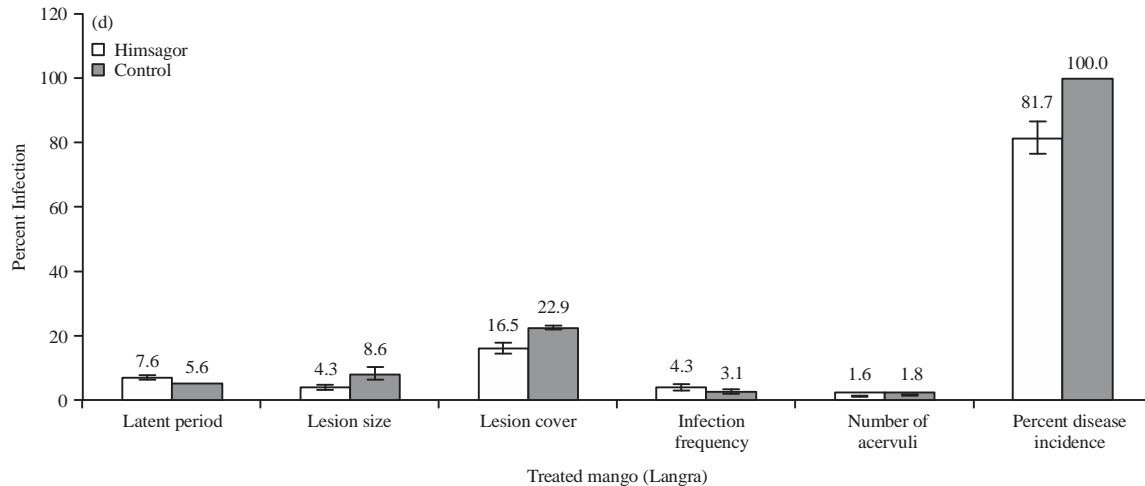


Fig. 4(a-d): Effectiveness and percent inhibition on mango by *Arsenicum album* Q at 10000 ppm/L concentration, (a) Treated mango (i-iii) Symptom developed after 7, 14, 18 days, (iv-v) Lesion length and width, (vi) Deeping mangoes, (vii-ix) Langra, Amrapali and Himsagar variety, (b-d) Graphs showing percent inhibition

CONCLUSION

A significant inhibitory effect (96.40%) of *Arsenicum album* Q at 10000 ppm on *C. gloeosporioides* was found. It was modestly effective on anthracnose of *Mangifera indica* L. caused by *C. gloeosporioides*. Thus, the use of homeopathic medicines may suggest their potential for controlling anthracnose of mango and other fruits.

SIGNIFICANCE STATEMENT

There is a need to focus on consumer and environment friendly, enduring and efficient bio-control method for management of anthracnose of *Mangifera indica* L. Effectiveness of homeopathic medicines have antifungal activities against *C. gloeosporioides*. For the choice of homeopathic drugs reduces the risk of health hazards, especially on mango for fresh fruit consumption.

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