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# Evaluation of different concentration of plant extracts as seed treatment to manage seed mycoflora of okra

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**Abstract**
Different concentration of four plant extracts viz. neem, garlic, *Cassia tora*, and ginger extract (10%, 20%, 30% and 40%) were used for testing the efficacy of plant extract against seed mycoflora of okra. In case of treatment with neem extract germination was recorded highest 81.40 % followed by 75.70 %, 62.80% and lowest was 49.28 % in the concentration of 40%, 30%, 20% and 10 % respectively, The seed treated with different plant extract, neem extract was recorded with minimum (29.61 %) frequency of mycoflora followed by *Cassia tora* extract (32.08 %), ginger extract (32.28 %) and maximum frequency was found in garlic extract (33.53 %). On an average among all the plant extract frequency of mycoflora was highest of *Fusarium sp.* (15.69 %) followed by *Aspergillus flavus* (13.77 %), *Alternaria sp.* (2.57 %), Sterile mycelium (0.89 %), *Chaetomium sp.* (0.76 %), *Curvularia sp.* (0.53 %), *Nigrospora sp.* (0.28 %) and lowest was *Rhizopus sp.* (0.08%). *Memnoniella sp.* was not found in all plant extracts treated seed.

**Keywords:** Plant extracts, ginger extract, seed mycoflora, garlic extract, seed germination, neem extract, *Cassia tora* extract

## Introduction
Okra (*Abelmoschus esculentus* L. Moench) is a familiar and famous vegetable grown in oriental areas especially in Indian subcontinent. Okra or lady's finger is locally known as "Dherosh" or "Bhendi" which belongs to the family Malvaceae. Nutritional profile of okra showed that it contains saturated fats, carbohydrates, proteins, vitamin A, B6, B12, folate, ribofalvin, niacin, pentothenic acid, Vitamin C, and E etc., it also contains magnesium, phosphorous, potassium, zinc, sodium, copper, manganese and selenium. The seeds also contains dietary fiber and sugars (Anon., 2012) [4]. The mucilage from okra is suitable for industrial and medicinal application and could be applied as plasma replacement or blood volume expander (Arapitsas, 2008) [5]. The leaf buds and flowers are also edible. The seed when roasted and ground can be used as coffee additive or substitute. Various factors are responsible for low yield of okra. Seed-borne fungal diseases are often the main cause. In most regions of the world, okra crop is produced in large quantities, poor agronomic practices and storage conditions including improper drying and inadequate structures have contributed to the reportedly high prevalence of fungal contaminants of okra especially seed-borne molds. So, management of these seed-borne fungi is very important to produce okra successfully. As there is no resistant variety, so control of these fungi through host resistance in not possible. Again control of these seed-borne fungi using chemicals increase production cost and causes environmental pollution. Plant extracts had shown good results as seed treating agent. Considerable amount of study have been done with chemical fungicide to control seed-borne disease of okra (Akter, 2008 and Ahmed, 2011) [2, 1]. But a few studies were done to control the seed-borne fungi of okra using plant extracts. For these reasons, three plant extracts have been used in this experiment viz. garlic extract, ginger extract, *Cassia tora* extract and neem extracts as seed treating agent.

## Materials and Methods
The experiment was conducted during the period of Kharif and Rabi season of 2016-17. For the testing of efficacy of plant extract following plants was used for seed treatment

*Azadirachta indica, Allium sativum, Cassia tora and Zingiber officinale*. The collected plant parts were chopped after cleaning under running tap water. The extracts were prepared by crushing the plant parts in a blender or mortar and pestle with distilled water at 1:2 (100 g crushed plant materials in 200 ml water). Later extract was boiled for 15 minuts then properly shaked with shaker. The extracts were filtered through cheese cloth and kept in conical flask. The extracts thus obtained were kept in a refrigerator at 4±1ºC until use. Seed samples were treated following dipping method. The seeds were dipped into previously prepared dose of neem, garlic, sickle pod and ginger extracts at the rate of 10, 20, 30, and 40 % concentration (for the making of different concentration sterilized water was used with plant extract). After proper covering of the seed coat with the extracts the remaining examined plants extracts were drained out from the petridishes and seeds were dried in air for some time by keeping on sterilized blotter paper. After incubating the treated seeds, germination of seeds were counted and association of mycoflora with germinated seed was recorded. The treated seeds were examined following the standard blotter method (ISTA, 1999; Neergaard, 1979 and Richardson, 1990) [11, 15, 17].

## Results and Discussion

### Effect of different concentration of plant extracts on germination of okra seeds

As germination of the seed is of major concern, it was observed that the treated seeds showed significantly higher rate of germination (Table 1). From the results, it was also observed that all the extracts increased the percentage of seed germination significantly. In case of treatment with neem seed extract, germination was recorded highest 81.40 % followed by 75.70 %, 62.80% and lowest 49.28 % in the concentration of 40%, 30%, 20% and 10 % respectively; in garlic extract seed germination was highest 82.80 % in 40 % concentration followed by 68.50%, 60% and lowest germination 45 % in 30 %, 10 % and 20 % of concentration, in *Cassia tora* extract germination was highest 62.80 % in 40 % of concentration followed by 62.80 %, 61.40 % and 59.20 % germination in 20 %, 10 % and 30 % of concentration and in ginger extract maximum 62.80 % germination was found in 30 % concentration followed by 58.80 %, 49.20 % and 47.80 % germination was found in 40 % 20 % and 10 % of concentration. In context of all the extract average highest germination was recorded in neem seed extract (67.29 %) followed by garlic (64.07 %), Cassia tora (61.55 %), and lowest germination was in ginger extract (54.65 %). Similarly in case of all concentration highest (71.45 %) germination was observed in 40 % concentration followed by 66.55 %, 54.95% and lowest germination 54.62 % in 30%, 20% and 10 % concentration. Similar results were reported by some earlier workers (Fakir, 1977; Neergaard, 1979; Fakir, 1982; Gupta et al., 1989; Fakir, 2000 and Jamandar et al., 2001) [8, 15, 7, 6, 9, 13].

**Table 1:** Effect of different concentration of plant extracts on germination of okra seeds

| Concentration (%) | Plant extracts | | | | Mean |
|---|---|---|---|---|---|
| | Neem | Garlic | *Cassia tora* | Ginger | |
| | (Germination %) | | | | |
| 10 | 49.28 | 60 | 61.4 | 47.80 | 54.62 |
| 20 | 62.80 | 45 | 62.8 | 49.20 | 54.95 |
| 30 | 75.70 | 68.50 | 59.2 | 62.80 | 66.55 |
| 40 | 81.40 | 82.80 | 62.8 | 58.80 | 71.45 |
| Control | 45 | 40 | 57 | 48 | 47.5 |
| **Mean** | 67.29 | 64.07 | 61.55 | 54.65 | |

### Effect of plant extracts in reducing seed-borne infection of okra seeds

The seed treated with neem seed extract was recorded with minimum frequency (29.61%) viz. *Fusarium sp.*(11.23%), *Aspergillus flavus* (12.13%), *Chaetomium sp.*(1.60%), *Nigrospora sp.*(1.15%), *Alternaria sp. (*2.49%), *Rhizopus sp.* (0.34%) and sterile mycelium (0.89%), and maximum frequency was recorded in garlic extract treated seed (33.53%) viz. *Fusarium sp.*(13.26%), *Aspergillus flavus* (13.74%), *Chaetomium sp.*(0.84%%), *Alternaria sp. (*1.42%), *Curvularia* (0.71%) and sterile mycelium (1.64%). In case of *Fusarium sp.* highest frequency was 26.18 % in *Cassia tora* extract followed by 13.26 % in garlic extract, 12.12% in ginger extract and lowest was 11.23% in neem extract, *Aspergillus flavus* was highest15.15% in *Cassia tora* extract followed by 14.08% in ginger extract, 13.74% in garlic extract and lowest was 12.13 % in neem extract, *Chaetomium sp.*was highest 1.60% in neem extract followed by 0.84 % in garlic extract, 0.44% in ginger extract and lowest was 0.17% in *Cassia tora* extract, *Alternaria sp.*was highest 3.61% in ginger extract followed by 2.76% in *Cassia tora* extract, 2.49% in neem extract and lowest was 1.42% in garlic extract, Sterile mycelium was highest1.64% in garlic extract followed by 0.89% in neem extract and lowest was 0.53% and 0.53% in *Cassia tora* and ginger extract, *Curvularia sp.*was found highest 1.07% in ginger extract, 0.71% in garlic extract and lowest 0.35% was in *Cassia tora* extract and not found in neem treated seed *Nigrospora sp*. and *Rhizopus sp.*was found only in neem treated seed with 1.15% and 0.34% respectively, *Memnoniella sp.*was not found in any plant extract treated seed while this was observed in control with 0.98% frequency. (Table 2)

Present results were supporting the findings of earlier researchers Khaleduzzaman (1996) [14], and Islam (2004) [12] in which an attempt has been made to control the fungi by different plant extracts. Among 7 plant extracts the extracts of gada and neem were found better.

**Table 2:** Effect of botanical extract on seed borne mycoflora of Okra

| Botanicals | Recorded mycoflora (%) | | | | | | | | | Frequency (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Fusarium sp.* | *Aspergillus flavus* | *Chaetomium sp.* | *Nigrospora sp.* | *Alternaria sp.* | *Rhizopus sp.* | *Curvularia sp.* | Sterile mycelium | *Memnoniella sp.* | |
| Neem | 11.23 | 12.13 | 1.60 | 1.15 | 2.49 | 0.34 | - | 0.89 | - | 29.61 |
| Garlic | 13.26 | 13.74 | 0.84 | - | 1.42 | - | 0.71 | 1.64 | - | 33.53 |
| *Cassia tora* | 26.18 | 15.15 | 0.17 | - | 2.76 | - | 0.35 | 0.53 | - | 32.08 |
| Ginger | 12.12 | 14.08 | 0.44 | - | 3.61 | - | 1.07 | 0.53 | - | 32.28 |
| Control | 27.02 | 22.77 | 6.43 | 3.35 | 13.21 | 2.46 | 4.02 | 9.02 | 0.98 | 89.26 |
| **Mean** | 15.69 | 13.77 | 0.76 | 0.28 | 2.57 | 0.08 | 0.53 | 0.89 | 0 | |

**Efficacy of different concentration of neem extracts on seed mycoflora of okra**

In the present experiment seed treatment with neem seed extract frequency of mycoflora was highest (32.84 %) in 10 % concentration viz. *Fusarium sp.* (15 %), *Aspergillus flavus* (10.71%), *Chaetomium sp.*(2.14 %), *Nigrospora sp.*(2.85 %), *Alternaria sp.*(2.14 %), and lowest (24.89 %) frequency of mycoflora was recorded in 40 % concentration of neem viz. *Fusarium sp.* (9.2%), *Aspergillus flavus* (12.85 %), *Chaetomium sp.*(1.42 %), and *Alternaria sp.* (1.42%). (Table.3)

In case of *Fusarium sp.*highest frequency was 15 % in 10 % concentration, 10 % in 20 % concentration and lowest 9.2 % frequency was found in 40 % concentration. *Aspergillus flavus* was highest 12.85 % in 40 % concentration followed by 15 % in 20 % concentration, 10.71% in 10 % concentration and lowest 10.70 % frequency was found in 30 % of concentration. *Chaetomium sp.* was highest 2.14 % in 10 % concentration and 1.42 % frequency was found in rest of all concentration. *Alternaria sp.* was highest 3.57 % in 30 % concentration followed by 2.85 % in 20 % concentration, 2.14 in 10 % concentration and lowest 1.42 % frequency was in 40 % concentration. *Nigrospora sp.*was found 2.85 % in 10 % concentration and 1.42 % in 20 % concentration and not found in 30 % and 40 % concentration *Rhizopus sp.* (1.42 %) was found only in 20 % concentration and sterile mycelium (3.57 %) was found only in 30 % concentration. On an average highest association of mycoflora in all concentration was *Aspergillus flavus* (12.31%) and lowest was *Rhizopus sp.* (0.33%). In control condition frequency of *Fusarium sp.* (24.29 %) was highest followed by *Aspergillus flavus* (21.43 %), *Alternaria sp.* (12.86 %), Sterile mycelium (8.75 %), *Rhizopus sp.*(7.14%), *Nigrospora sp.* (7.14 %) and lowest was *Chaetomium sp.*(5.71 %) (Table 3). On an average among all the plant extract frequency of mycoflora was highest of *Fusarium sp.* (15.69 %) followed by *Aspergillus flavus* (13.77 %), *Alternaria sp.*(2.57 %), Sterile mycelium (0.89 %), *Chaetomium sp.*(0.76 %), *Curvularia sp.*(0.53 %), *Nigrospora sp.* (0.28 %) and lowest was *Rhizopus sp.*(0.08%). *Memnoniella sp.* was not found in all plant extracts treated seed. This result corroborates with the findings of Ambekar *et al.* (2000) [3], Zaman *et al.* (1997) [21], Hossain (2001) [10], Singh and Kumar (2003) [19].

**Table 3:** Efficacy of different concentration of neem extract on seed mycoflora of okra

| Concentration (%) | Recorded mycoflora (%) | | | | | | | Frequency (%) |
|---|---|---|---|---|---|---|---|---|
| | *Fusarium sp.* | *Aspergillus flavus* | *Chaetomium sp.* | *Nigrospora sp.* | *Alternaria sp.* | *Rhizopus sp.* | Sterile mycelium | |
| 10 | 15 | 10.71 | 2.14 | 2.85 | 2.14 | - | - | 32.84 |
| 20 | 10 | 15 | 1.42 | 1.42 | 2.85 | 1.42 | - | 32.11 |
| 30 | 10 | 10.7 | 1.42 | - | 3.57 | - | 3.57 | 29.26 |
| 40 | 9.2 | 12.85 | 1.42 | - | 1.42 | - | - | 24.89 |
| Control | 24.29 | 21.43 | 5.71 | 7.14 | 12.86 | 7.14 | 8.75 | 85.71 |
| **Mean** | 11.05 | 12.31 | 1.6 | 1.06 | 2.49 | 0.33 | 0.89 | 29.77 |

In seed treatment with garlic extract, the frequency of mycoflora was highest (38.55%) in 20% concentration viz. *Fusarium sp.*(15 %), *Aspergillus flavus* (15 %), *Chaetomium sp.*(2.85%), *Alternaria sp.*(1.42 %), Sterile mycelium (4.28 %), and lowest (31.72 %) frequency was in 30 % concentration viz. *Fusarium sp.*(12.8%), *Aspergillus flavus* (13.57 %), *Chaetomium sp.*(2.5 %), *Alternaria sp.*(2.85%).

In case of *Fusarium sp.* highest frequency was 15 % in 20 % concentration followed by 13.5 % in 40 % concentration, 12.8 % in 30 % concentration and lowest was 10.7 % in 10 % concentration, *Aspergillus flavus* was highest 15 % in 20 % concentration followed by 13.57 % in 30 % and 40 % concentration and lowest was 12.8 % in 10 % concentration, *Alternaria sp.* was highest 4.28 % in 10 % concentration followed by 2.85 % in 30 % and 40 % concentration and lowest was 1.42 % in 20 % concentration, *Chaetomium sp.* was highest 2.85 % in 20 % concentration, 2.5 % in 30 % concentration and lowest 1.42 % in 10 % concentration and not found in 40 % concentration, Sterile mycelium was highest 4.28 % in 20 % concentration and lowest was 2.5 % in 40 % concentration and not found in 10 % and 30 % concentration, *Curvularia sp.* was recorded only in 10 %

concentration *Memnoniella sp.* was not found in any concentration while this was 2.86 % in control condition.
Highest frequency of mycoflora in control condition was *Fusarium sp.* (25.71 %) followed by *Aspergillus flavus* (21.43 %), *Alternaria sp.* (12.84 %), Sterile mycelium (8.57 %), *Chaetomium sp.* (7.14 %), *Curvularia sp.* (5.71 %) and lowest was *Memnoniella sp.* (2.86 %). On an average highest associated mycoflora among all concentration was *Aspergillus flavus* (13.73 %) followed by *Fusarium sp.* (13.5 %), *Alternaria sp.* (2.85 %), *Chaetomium sp.* and sterile mycelium both was (1.69 %) and lowest was *Curvularia sp.* (0.71%), (Table 4).

Seed soaking in different concentration (%) in garlic extract extract did not show any significance in controlling microbial frequencies.
Similar results were reported by (Akter, 2008) [2]. However, garlic extract used in controlling seed-borne infection of different crops showed that garlic extract was a potential agent to control the seed-borne pathogens of different vegetable crops (Zaman *et al*., 1997 and Hossain, 2001) [21, 10]. It is also corroborated the result in case of other crops (Rahman *et al*., 1999 Sultana, 2009) [16, 20].

**Table 4:** Efficacy of different concentration of garlic extract on seed mycoflora of okra

| Concentration (%) | Recorded mycoflora (%) | | | | | | | Frequency (%) |
|---|---|---|---|---|---|---|---|---|
| | *Fusarium sp.* | *Aspergillus flavus* | *Chaetomiumsp.* | *Alternaria sp.* | Sterile mycelium | *Curvularia sp.* | *Memnoniella sp.* | |
| 10 | 10.7 | 12.8 | 1.42 | 4.28 | - | 2.85 | - | 32.05 |
| 20 | 15 | 15 | 2.85 | 1.42 | 4.28 | - | - | 38.55 |
| 30 | 12.8 | 13.57 | 2.5 | 2.85 | - | - | - | 31.72 |
| 40 | 13.5 | 13.57 | - | 2.85 | 2.5 | - | - | 32.42 |
| Control | 25.71 | 21.43 | 7.14 | 12.84 | 8.57 | 5.71 | 2.86 | 84.29 |
| **Mean** | 13.5 | 13.73 | 1.69 | 2.85 | 1.69 | 0.71 | - | 33.68 |

In *Cassia tora* extract treated seed, the frequency of mycoflora was highest (37.14%) in 10 % concentration viz. *Fusarium sp.*(13.5%), *Aspergillus flavus* (17.8%), *Alternaria sp.*(3.57%), and lowest (27.14%) frequency was in 40 % concentration viz. *Fusarium sp.*(11.4%), *Aspergillus flavus* (12.85%), *Alternaria sp.*(2.85%).
In case of *Aspergillus flavus*, highest frequency was 17.8 % in 10 % concentration followed by 15.7 % in 30 % concentration, 14.2 % in 20 % concentration and lowest was 12.85 % in 40 % concentration, *Fusarium sp.* was highest 14.2 % in 20 % concentration followed by 13.5 % in both 10 % and 30 % concentration and lowest frequency was 11.4 % in 40 % concentration, *Alternaria sp.* was highest 3.57 % in both 10 % an 30 % concentration followed by 2.85 % in 40 % concentration and lowest was 2.14 % in 20 % concentration, *Chaetomium sp.* (0.71 %) was found only in 30 % concentration, *Curvularia sp.* (2.14 %) was found only in 10 % concentration and sterile mycelium was found only in 20 % concentration.
*Fusarium sp.* was highest (22.86 %) in control condition followed by *Aspergillus flavus* (20 %), *Alternaria sp.* (14.29 %), sterile mycelium (10 %), *Curvularia sp.* (7.14 %) and lowest was *Chaetomium sp.* (5.71 %). Among all concentration average higher prevalence was *Aspergillus flavus* (15.13%) followed by *Fusarium sp.* (13.15 %), *Alternaria sp.*(3.03 %), *Curvularia sp.* and sterile mycelium both was (0.53 %) and lower was *Chaetomium sp.* (0.17%),(Table 5).

**Table 5:** Efficacy of different concentration of *Cassia tora* extract on seed mycoflora of okra

| Concentration (%) | Recorded mycoflora (%) | | | | | | Frequency (%) |
|---|---|---|---|---|---|---|---|
| | *Aspergillus Flavus* | *Fusarium sp.* | *Alternaria sp.* | *Chaetomium sp.* | *Curvularia sp.* | Sterile mycelium | |
| 10 | 17.8 | 13.5 | 3.57 | - | 2.14 | - | 37.14 |
| 20 | 14.2 | 14.2 | 2.14 | - | - | 2.14 | 31.42 |
| 30 | 15.7 | 13.5 | 3.57 | 0.71 | - | - | 33.57 |
| 40 | 12.85 | 11.4 | 2.85 | - | - | - | 27.14 |
| Control | 20 | 22.86 | 14.29 | 5.71 | 7.14 | 10 | 81.43 |
| **Mean** | 15.13 | 13.15 | 3.03 | 0.17 | 0.53 | 0.53 | 32.56 |

In ginger extract treated seed the frequency of mycoflora was highest (37.08%) in 10 % concentration viz. *Fusarium sp.* (12.8%), *Aspergillus flavus* (15%), *Alternaria sp.* (5.71%), *Chaetomium sp.* (3.57%), and lowest (25%) frequency was in 40 % concentration viz. *Fusarium sp.* (11.4%), *Aspergillus flavus* (11.4%), *Alternaria sp.*(2.85%). (Table 6).

*Aspergillus flavus* was highest (15.7 %) in 30 % concentration followed by 15 % in 10 % concentration, 14.2 % in 20 % concentration and lowest was 11.4 % in 40 % concentration, *Fusarium sp.* was highest 12.8 % in both 10 % and 20 % concentration and lowest was 11.4 % in 30 % and 40 % concentration. *Alternaria sp.* was highest 5.71 % in 10 %

concentration followed by 4.28 % in 30 % concentration, 2.85 % in 40 % concentration and lowest was 2.14 % in 20 % concentration. *Chaetomium sp.* (3.57 %) was found only in 10 % concentration, *Curvularia sp.* (3.57 %) was found only in 20 % concentration and sterile mycelium (2.14 %) was found only in 30 % concentration.

In control condition *Fusarium sp.* (30 %) was found with highest frequency followed by *Aspergillus flavus* (24.9 %), *Alternaria sp.*(15.7 %), sterile mycelium (7.14 %), *Curvularia sp.* (4.29 %) and lowest was *Chaetomium sp.* (2.86%). On an average among all concentration *Aspergillus flavus* (14.07%) was highest frequency followed by *Fusarium sp.*(12.1 %), *Alternaria sp.*(3.74 %), *Chaetomium sp.* and sterile mycelium both was (0.89 %) and sterile mycelium (0.53%) was associated with lowest frequency in seed (Table 6). Similar result was observed by Saha *et al.* (2014) [18].

Seed soaking in different concentration (%) in ginger extract did not show any significance in controlling microbial frequencies.

**Table 6:** Efficacy of different concentration of ginger extract on seed mycoflora of okra

| Concentration (%) | Recorded mycoflora (%) | | | | | | Frequency (%) |
|---|---|---|---|---|---|---|---|
| | *Aspergillus flavus* | *Fusarium sp.* | *Alternaria sp.* | *Chaetomium sp.* | *Curvularia sp.* | *Sterile mycelium* | |
| 10 | 15 | 12.8 | 5.71 | 3.57 | - | - | 37.08 |
| 20 | 14.2 | 12.8 | 2.14 | - | 3.57 | - | 32.71 |
| 30 | 15.7 | 11.4 | 4.28 | - | - | 2.14 | 33.52 |
| 40 | 11.4 | 11.4 | 2.85 | - | - | - | 25.65 |
| Control | 24.29 | 30 | 15.71 | 2.86 | 4.29 | 7.14 | 84.29 |
| **Mean** | 14.07 | 12.1 | 3.74 | 0.89 | 0.89 | 0.53 | 32.24 |

Among all concentration of plant extract association of *Fusarium sp.* (9.2%) was lowest in 40 % concentration of neem extract, *Aspergillus flavus* (10.7%) was lowest in 30 % concentration of neem extract, *Chaetomium sp.* (0.71%) was lowest in 30 % concentration of *Cassia tora* extract, *Curvularia sp.* (2.14%) was lowest in 10 % concentration of *Cassia tora* extract, *Alternaria sp.* (1.42%) was lowest in both 20 % concentration of garlic extract and 40 % neem extract, *Rhizopus sp.* (1.42%) was in 20 % concentration of neem extract and sterile mycelium (2.14 %) was lowest in both 30 % concentration of ginger extract and 20 % concentration of *Cassia tora* extract.

Seed soaking in different concentration did not show any significance in controlling microbial frequencies. In average neem extract was found best for seed treatment followed by garlic.

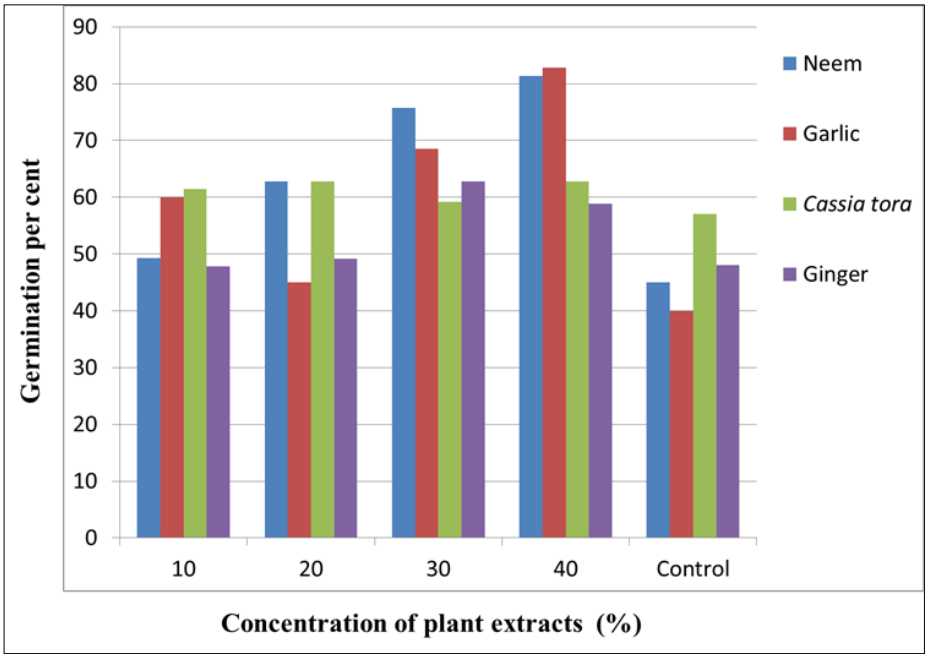


**Fig 1:** Effect of different concentration of plant extracts on seed germination

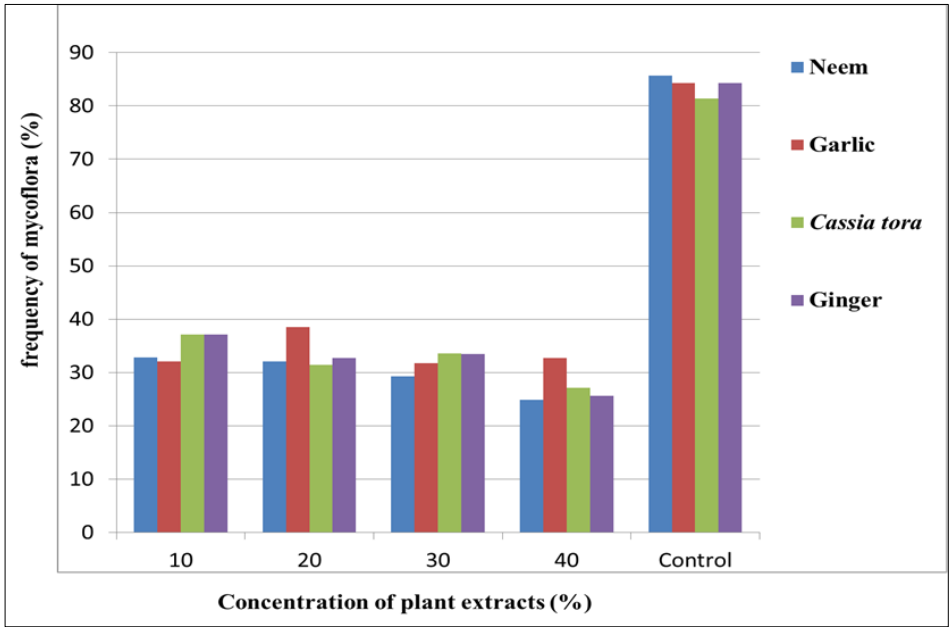


**Fig 2**: Effect on frequency of mycoflora on different concentration of plant extracts

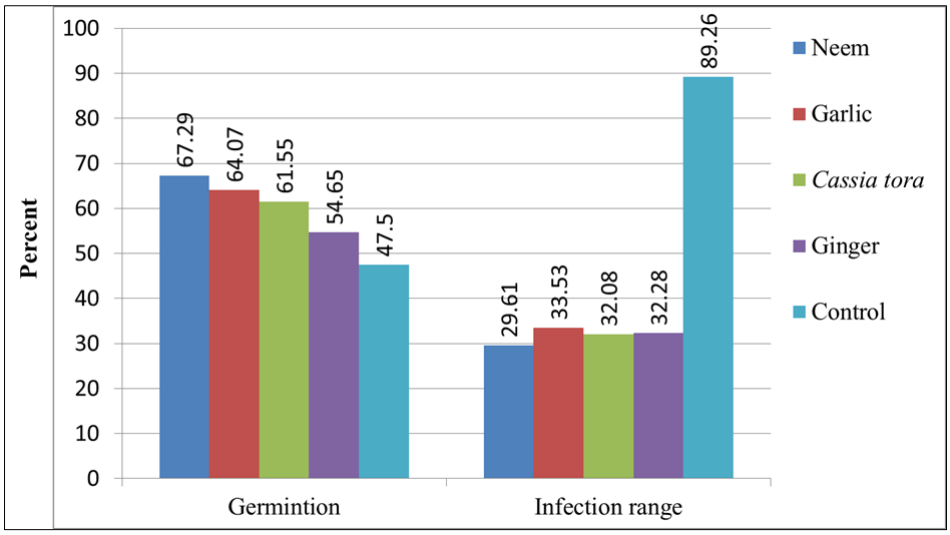


**Fig 3:** Effect of botanical extract on seed germination and infection range of mycoflora of okra

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