Nematicidal and Nematostatic activity of plant extracts on Heterodera spp.

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Abstract

The bark extracts of *Ficus bengalensis* and *Eugenia jambolana* were implied to understand the nematicidal and nematostatic activity against Heterodera sp. The study showed that the extracts were found to be both nematicidal and nematostatic and cause immobility of the juveniles. This finding is significant from the viewpoint of controlling cereal cyst nematodes affecting cereal crops without the use of chemical nematicides which causes environmental hazards.

Keywords: *Ficus bengalensis*, *Eugenia jambolana*, Heterodera, Nematicidal, Nemtostatic.

Introduction:

Grain crops are constantly under consistent stress because of parasitic attack during plant development. Among parasites, nematodes are one of the most significant parasitic gatherings which decrease the grain crop yield around the world. Nematodes are soil-borne parasites which are additionally called roundworms with complex body frameworks and happen overall any place wherever cereals are produced. These parasites assault harvests and cause evaluated yearly loss of $ 78 billion around the world [1]. There are a few sorts of nematodes that attack the yields. These are stunt and pin nematodes (*Merlinius brevidens*), RLN (*Pratylenchus neglectus* and *P. thornei*), RKN (*Meloidogyne spp.*) and CCN (*Heterodera spp.*). Among these, Heterodera is the class called as CCN and cause more prominent harm to the grain crops. The cereal cyst nematode nematode was first found as a parasite of cereals in Germany and is presently detailed in most wheat developing territories of the world [2]. The genus Heterodera is the earliest known genera of plant parasitic nematode (PPN). *Heterodera avenae*, *H. latipons*, and *H. filipjevi* are viewed as the most financially significant species in grains around the world. The misfortune because of the parasitic disease can be limit by utilizing diverse control systems viz., manufactured synthetics, bio-control specialists, and customary strategies and by utilizing concentrates of plants.

Chemical control of nematodes is the best strategy however the issue is that they are costly to utilize, poisonous to human and creatures, decimate the regular predators of nematodes in the nature and steady for quite a while in the earth. Natural alterations can be utilized as an effective way to deal with compound control of PPN yet increasingly point by point considers are required to affirm their anthelmintic properties [3]. There are beneficial effects of organic amendments on soil physical condition, nutrients, fertility and biological activities. Several compounds derived from plants are involved in plant-nematode interactions. These compounds include repellents, attractants, hatching stimulants or inhibitors and nemato-toxicants. These compounds are formed in response to nematode occurrence.

Numerous plants and their parts are accounted for to have poisonous substances to plant-parasitic nematodes. Distinctive plant removes are compelling in charge of plant-parasitic nematodes. These extracts prepared from both annual and perennial plants which belongs to 46 plant families [4, 5, 6]. Many plants synthesized compounds that can kill or keep away harmful organisms by destroying their lifecycles and acting as an attractant or repellent. Extracts of plants containing volatile compounds, essential oils have some anthelmintic properties. Volatile and non-volatile compounds are present in plant extracts and some of them can be detected as attractant or repellent effect on nematodes. Various plants and their parts have been used for nematicidal/ nematostatic activity- Siam weed (*Chromolaena odorata*), Neem, Castor bean (Ricinus communis) and Lemon grass [7], *Lantana camara* [8], *Chrysanthemum coronarium* and *Datura metel* [9], *Inula viscosa* (aqueous) and *Myrtus communis.* Therefore, it is necessary to examine new plants or new varieties of plants for their efficiency in immobilizing, retarding development or killing nematodes. Since, many other plants such as *Artemisia vulgaris, Azadirachta indica, Brassica napus, Cannabis sativa, Crotolaria juncea, Moringa oleifera, Myrtus communis* etc. are found to have anthelminthic properties and many more are yet to be discovered [10, 11, 12, 13].

Syzygium cumini (*Eugenia jambolana*) usually known as the Indian blackberry of family Myrtaceae [14]. S. cumini is an enormous evergreen tree and local to India. Various parts of Jamun plants, for example, bark, leaves, natural product, and seeds have been utilized in different conventional frameworks of medication [15]. Different concentrates of Jamun shows properties, for example, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, anti-allergic, anti-cancer, anti-oxidant, anti-diarrheal, and anti-diabetic [16]. The bark likewise shows anthelminthic properties [17] as well.

Ficus bengalensis is normally known as Banyan tree or 'Bargad' in Hindi and has a place with the family Moraceae. It is disseminated all through India and considered a sacrosanct tree [18]. This plant is utilized as a solution for skin infections and respiratory issues [17]. Bark has been utilized generally utilized for the control of diabetes mellitus [19]. Methanolic, fluid, chloroform, oil, ether concentrates of roots were accounted for to have anthelmintic action against earthworm [20]. Bark extracts (water, oil, ethanolic, methanolic) show anthelminthic, against unfavorably susceptible and wound recuperating properties [21].

Thus, the present study was designed to understand the anti helminthic property of extracts of *Syzygium cumini* and *Ficus bengalensis.*

Materials and Methods

Cysts Collection

The soil sample for *Heterodera* cysts collection was provided by Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana). The soil sample was washed under tap water and filtered through 150 mm sieve. The slurry remained in the bottom of sieve was collected in a beaker and some tap water added. The slurry was observed under stereo-microscope and the cysts were collected. Now the cysts were sterilized in 1% sodium hypochlorite solution and stored in Eppendorf tubes containing distilled water.

Plant Material:

The fully mature, fresh stem bark of *Eugenia jambolana* (EJ) and *Ficus bengalensis* (FB) was collected the botanical garden of Chaudhary Charan Singh Haryana Agriculture University, Hisar. The bark was air dried at room temperature and then with the help of an electric grinder converted into fine powder. The powder was kept in deep freezer until the time of use.

Preparation of Plant Extract:

For the preparation of plant extract 25 gm of dry fine powder was taken and soaked in 75 ml of distilled water for 24 hours. After filtering through two folds of muslin cloth, the extract was filtered through Whatman No. filter paper. The filtrate was centrifuged at 5000 rpm for 20 minutes and clear supernatant was stored at 4°C. This was considered as crude (100%) solution and other different concentrations (50%, 25%) was prepared from crude solution with adding requisite amount of distilled water at the time of experiment.

Nematicidal Bioassay:

The bioassay was performed at 25 ± °C, in 6-welled culture plates containing 3 ml/well, of different concentration (crude/100%, 50%, 25%) of aqueous plant extracts and 3 mM Zinc Sulphate solution was used as control. In each well 5 cysts of *Heterodera spp*. were added for the study. Each treatment was replicated three times. Emerged juveniles were removed and counted every week over a period of six-weeks. The solutions were renewed weekly. After three weeks the cysts were removed from the extracts and the incubation continued for the remaining three weeks in zinc sulphate solution. At the end of the experiment cysts were crushed and unhatched eggs and juveniles were counted. Nematodes were considered dead when there was a complete lack of motion. Number of second stage juveniles emerging weekly was expressed as cumulative percentage of the total egg content of the cysts. One-way analysis of variance (ANOVA) and t-test were applied at $p<0.01$ & <0.05.

Results:

Nematicidal effect of *F. bengalensis* (FB) & *E. jambolana* (EJ) bark extract on Heterodera spp. (HS):

All the tested plant bark extracts showed nematicidal effect against juveniles of HS. Extract of FB (100%) found to be very effective against juveniles (J2) of HS followed by 50% EJ extract (Fig. 2.1). Extract of EJ (25%) was found least effective against J2s of HS (Fig. 2.2). The mortality percentage of J2s was reported up to 85% when treated with 100% extract of FB and 78.2% in 50% bark extract EJ (Fig. 2.1 & 2.2). The mortality percentage of J2s was reported least in 25% EJ bark extract which was 39.3% (Fig. 2.2). Extracts of 100% EJ, 50% & 25% FB were also caused mortality of 69.18%, 61.4% & 59.85% of juveniles respectively. The result in 100% FB extract was significantly less ($p<0.01$ & 0.05) than the control (Fig. 2.1). Although, a significant mortality of juveniles was also reported in control ($ZnSO_4$) solution 62.3%. In comparative analysis, 100% FB extract was found more effective than 100% EJ. But the extract of 50% EJ was more effective than 50% FB. The mortality percentage of juveniles was recorded more in 50% EJ extract. The extract of 25% FB also showed more mortality of juveniles than in 25% EJ extract (Fig. 2.3).

Nematostatic effect of *F. bengalensis* (FB) & *E. jambolana* (EJ) bark extract on Heterodera spp. (HS):

Plant bark extracts showed nematostatic effect against juveniles of HS. The bark extract of 50% FB was found very effective against the juveniles of HS. The immobility (Nematostatic) percentage of J2s was reported 8.37% when treated with 50% FB bark extract and followed by 7.92% of 25% FB bark extract (Fig. 3.1). The bark extract of 100% EJ was reported to have 7.83% immobility of juveniles of HS. The immobility percentage of J2s was found least when treated with 50% EJ bark extract (Fig. 3.2). Other concentrations of bark extracts also showed significant immobility of J2s of HS. The immobility percentage of juveniles was reported 6.83% & 7.68% when treated with bark extracts of 100%FB and 25% EJ (Fig. 3.1 & 3.2). The result in 50% EJ extract was significantly less ($p < 0.05$) than the control (Fig. 3.2). Although, a significant immobility of J2s were also reported in control ($ZnSO_4$) solution 11.89%. In comparative analysis, immobility percentage of juveniles was recorded more in 100% EJ extract than in 100% FB. But the immobility percentage was found more in 50% FB extract than in 50% EJ. Nearly equal immobility percentage was recorded in 25% FB & 25% EJ extract (Fig. 3.3).

Effect of *F. bengalensis* (FB) & *E. jambolana* (EJ) bark extract on hatching Heterodera spp.(HS):

It was reported that, in all tested plant bark extract concentrations majority of eggs remain unhatched due to inhibition. Maximum inhibition of eggs was reported 95.6% when treated with 50% EJ bark extract followed by 93.1% in 100% FB extract (Fig. 4.1 & 4.2). The extract of 50% FB had the lowest percentage (88%) of inhibition on hatch of HS eggs (Fig. 4.1). Other concentrations of plant bark extract also showed significant inhibition to hatching of eggs of HS. When cysts of HS treated with 25% FB, 100% & 25% EJ bark extracts, 92%, 92.1% & 92.3% eggs hatch inhibition was reported (Fig. 4.1 & 4.2). The result of inhibition of egg hatch, in 50% EJ extract was significantly less ($p < 0.05$) than the control (Fig. 4.2). Although, 68% of eggs hatch inhibition was also reported in control ($ZnSO_4$). In comparative analysis, 100% FB extract was recorded with more inhibition of egg hatch than 100% EJ. But the inhibition was found more in 50% EJ extract than 50% FB. The inhibition of egg hatch was also reported more in 25% EJ than 25% FB extract (Fig. 4.3).

Discussion:

The members of Heterodera genus are obligate parasites of cereal crops like wheat, barley, oats etc. These PPN are found worldwide and cause significant economic yield losses in many countries [22]. So, there is a need to control these plant parasitic nematodes or cereal cyst nematodes. There is different control methods used to control these parasitic nematodes. The control methods are chemical methods, biological agents, and traditional methods and by using plant extracts. Chemical control of parasitic nematodes is the most effective method till today but there is a problem with them, is that they are hazardous to the environment, humans and other animals. Many plants and plant compositions are reported with antihelminthic properties. Some plants synthesize different compounds that can kill or keep away the harmful organisms. Plants like neem and Tagetes spp. are reported with significant effect against root-knot nematodes.

The nematicidal and nematostatic activity of *Ficus bengalensis* (EB) and *Eugenia jambolana*, (EJ) against the cereal cyst nematode, Heterodera spp. (HS) was evaluated in this experiment. In present study, it was reported that bark extracts from both plants were effective against the juveniles of HS. It caused a significant mortality of juveniles of HS, when treated with bark extracts of above mentioned plants. Nearly 80-85% mortality of juveniles of HS was reported when treated with the bark extract of FB & EJ. The highest percentage of juveniles mortality was reported in 100% FB & 50% EJ the bark extract. The least mortality percentage was reported in 25% EJ bark extract. As per the literature cited, several plant species were reported with nematicidal properties. Among them Neem and Tagetes spp. were reported with high mortality percentage of nematodes when treated with extracts prepared from different plant parts such as leaves, bark, root etc. [3, 23, 24].

It was reported that the bark extract from both the plants also caused immobility (Nematostatic) of juveniles of HS. Treatment with bark extract paralyzed the juveniles and affects their activity and movement. In present study, 50% and 25% FB bark extract showed 8.37% & 7.92% of immobility of juveniles of HS. The bark extract of 50% EJ was reported with least immobility percentage. As per the literature cited, several plant extracts were reported with nematostatic properties against juveniles of nematodes. *Chrysanthenam coromarium, Lantana camara* was reported with nematostatic properties against (root-knot nematodes) meloidogyne spp. [8, 25, 26]. Phyto-chemicals with suppressive effect on various nematode population has been well documented in several pathosystem [27].

Phytochemical investigation of *Ficus bengalensis* and *Eugenia jambolana* displays the existence of Tannins, Saponins, Phenols, Terpenoids, Phytosterols, Carbhohydrates, Flavonoids and Amino acids [28]. The aqueous extract of FB and EJ was reported with antihelminthic (nemticidal & nematostatic) activity due to the presence of above stated phyto-compounds. Polyphenolic compounds were reported with antihelmintic activity against different nematode species. Certain synthetic phenolic compounds disturb the oxidative phosphorylation mechanism of energy generation in nematodes and kill those [29]. Tannins are also reported with antihelmintic properties. Tannins kill the parasitic nematodes by binding to the free proteins in the gastro-intestinal tract or glycoproteins of cuticle [30, 31, 32]. Methanolic and aqueous bark extract of EJ which possess tannins also showed the similar effect against parasitic nematodes.

In this present study, the percentage hatch inhibition of eggs was reported high when the cysts of HS treated with the bark extract of FB and EJ. The bark extract of 50% EJ showed the maximum eggs hatch inhibition with a percentage of 95.6%. Other concentrations of both plants bark extracts also showed significant inhibition of eggs hatching. The least hatching inhibition was detected in ZnSO4 (Control) solution 68%. As per the literature cited, several plant extracts were reported to inhibit the hatching of eggs pf HS. The root extract of Siam weed, Neem were reported to show high inhibition of egg hatching against the RKN [7, 9].

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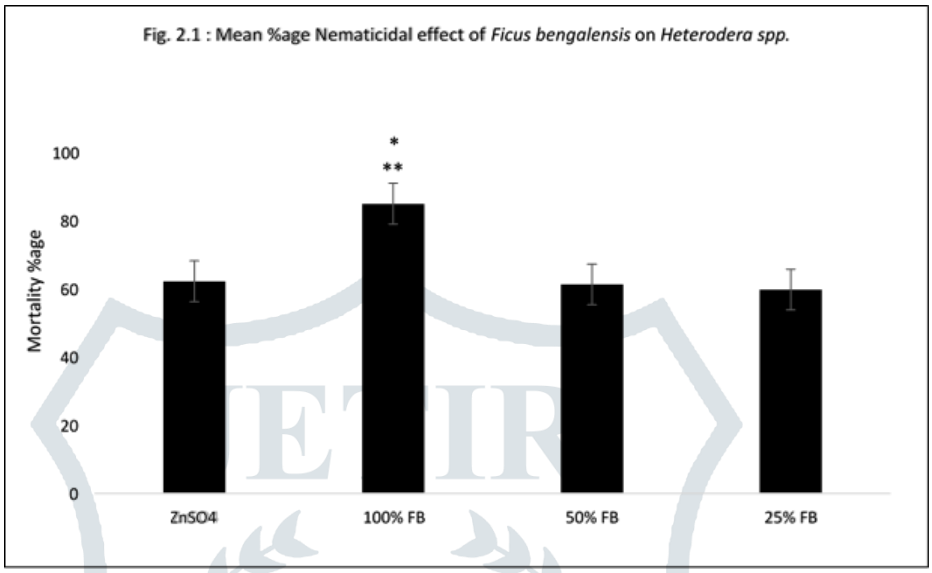
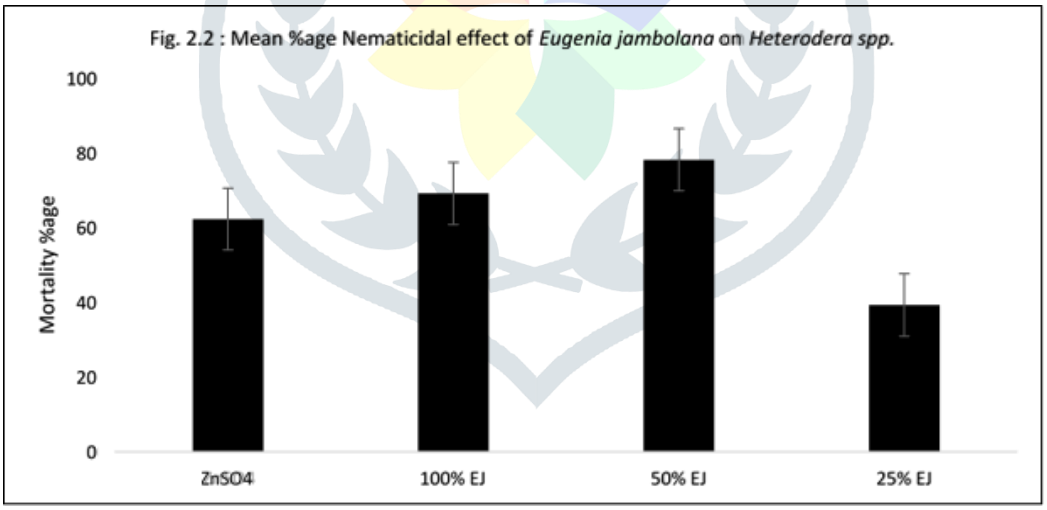


(* and ** is significant at p< 0.01 & 0.05)



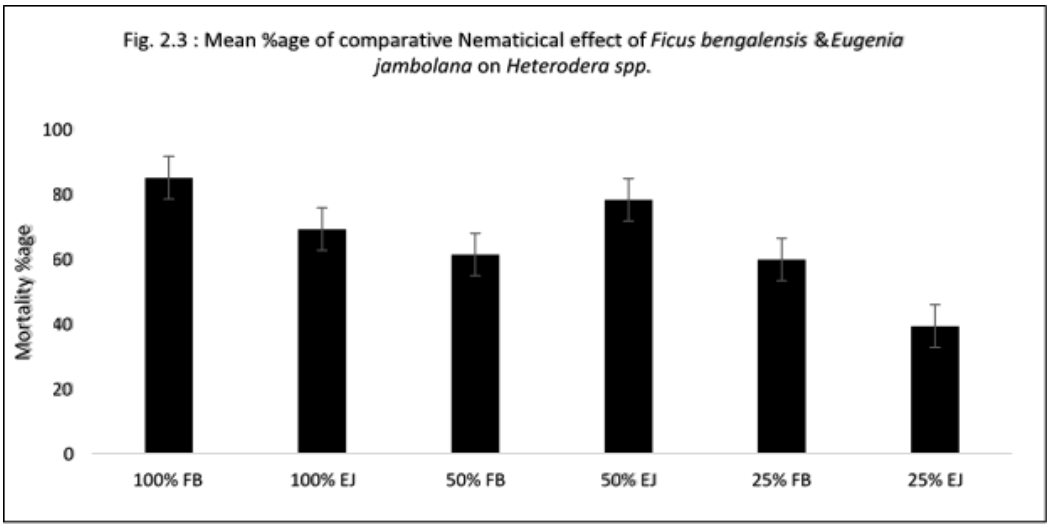


Fig. 2.3 : Mean %age of comparative Nematicical effect of Ficus bengalensis &Eugenia jambolana on Heterodera spp.
Mortality %age
100
80
60
40
20
0
100% FB
100% EJ
50% FB
50% EJ
25% FB
25% EJ

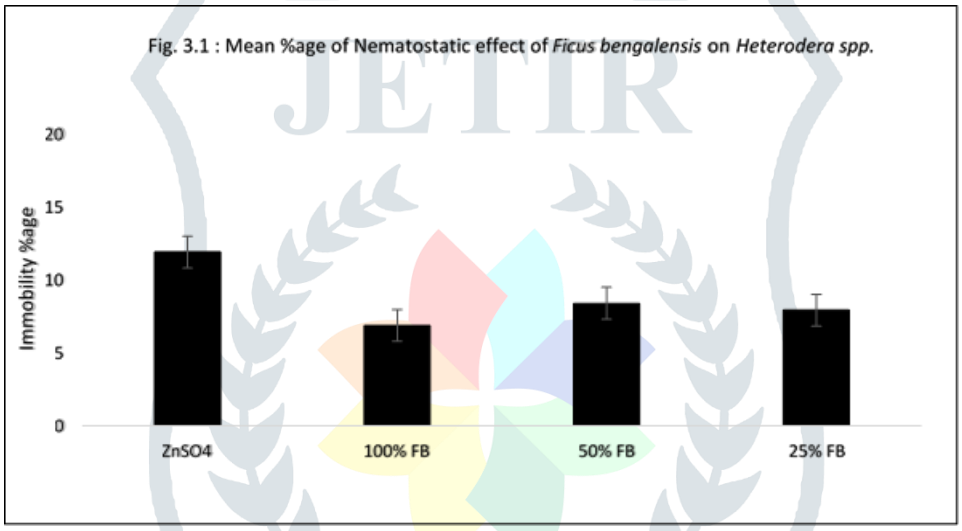


Fig. 3.1 : Mean %age of Nematostatic effect of Ficus bengalensis on Heterodera spp.
Immobility %age
20
15
10
5
0
ZnSO4
100% FB
50% FB
25% FB

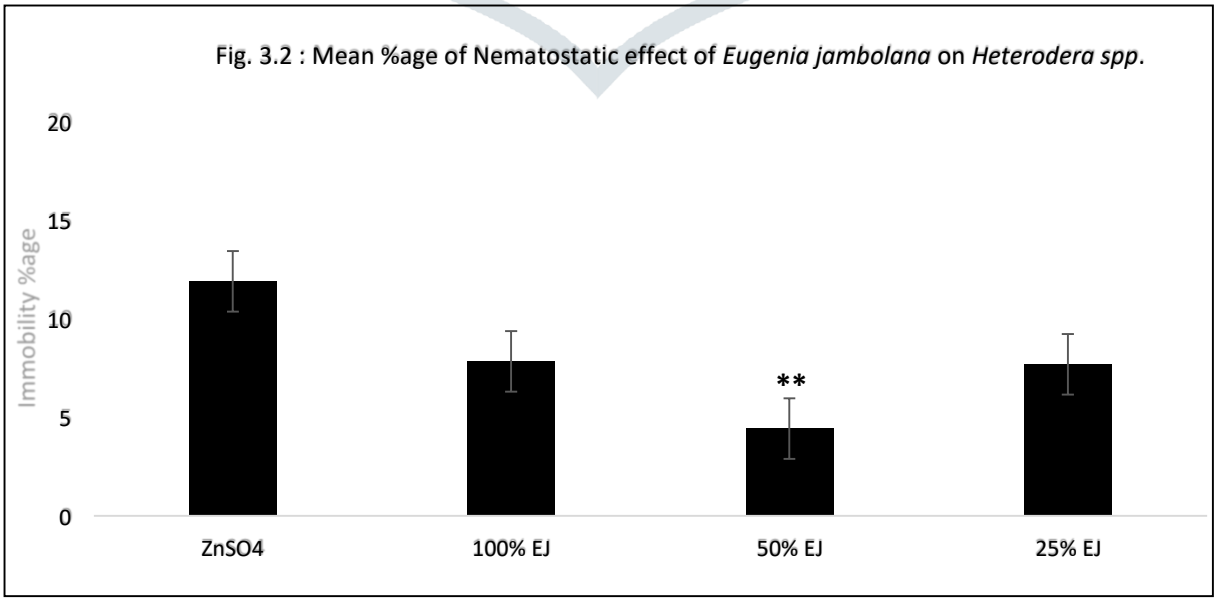
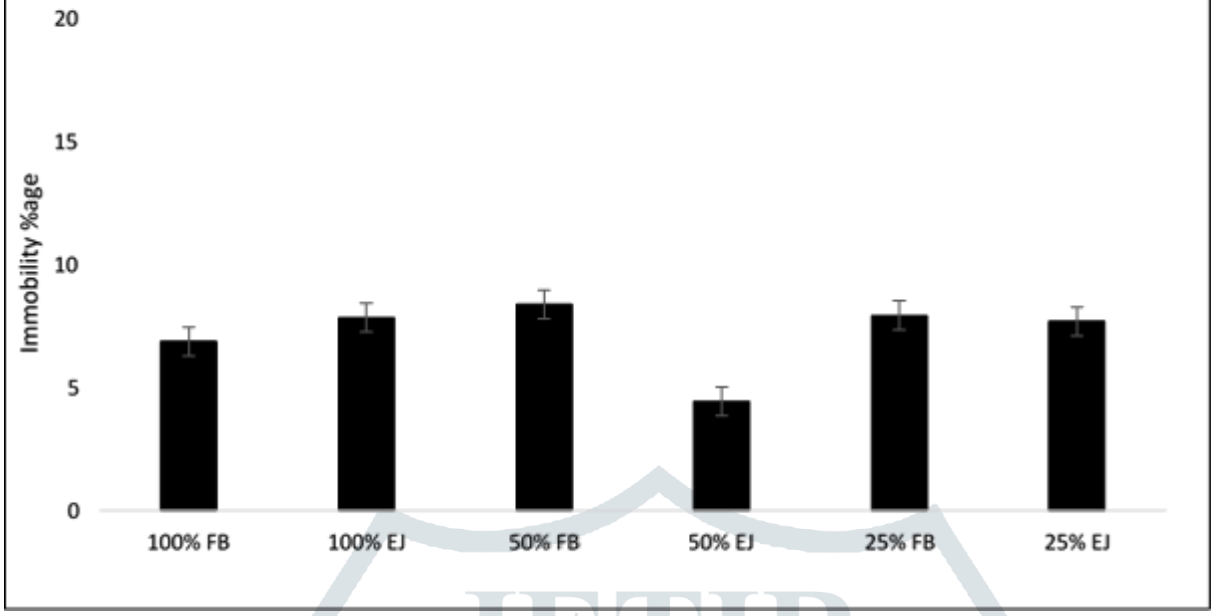


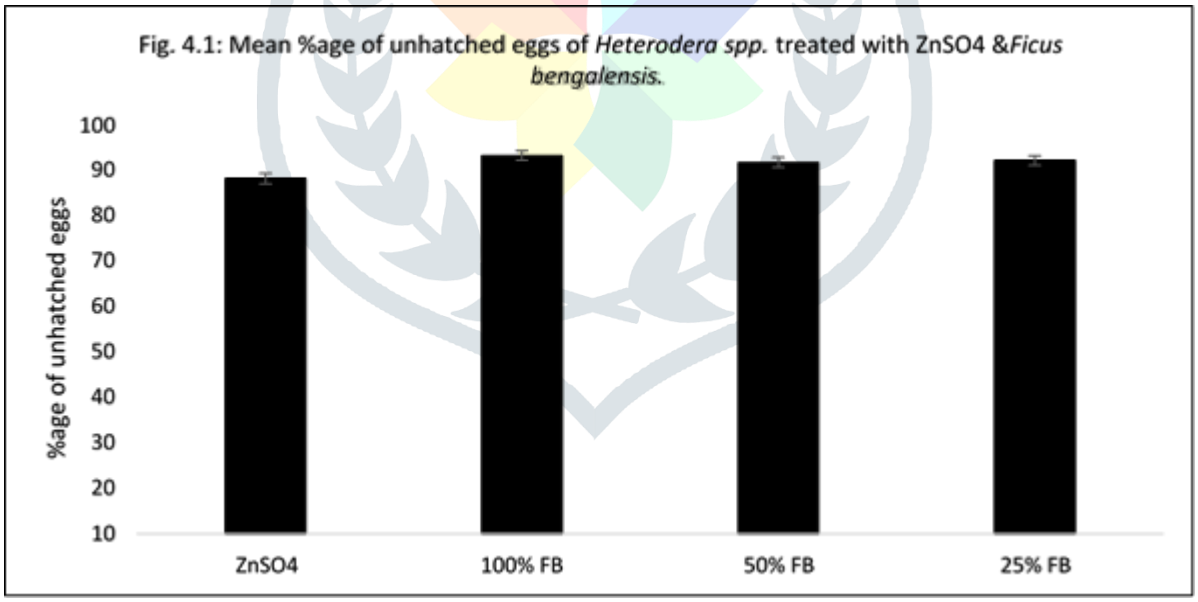
Fig. 3.2 : Mean %age of Nematostatic effect of Eugenia jambolana on Heterodera spp.
Immobility %age
20
15
10
5
0
**
ZnSO4
100% EJ
50% EJ
25% EJ

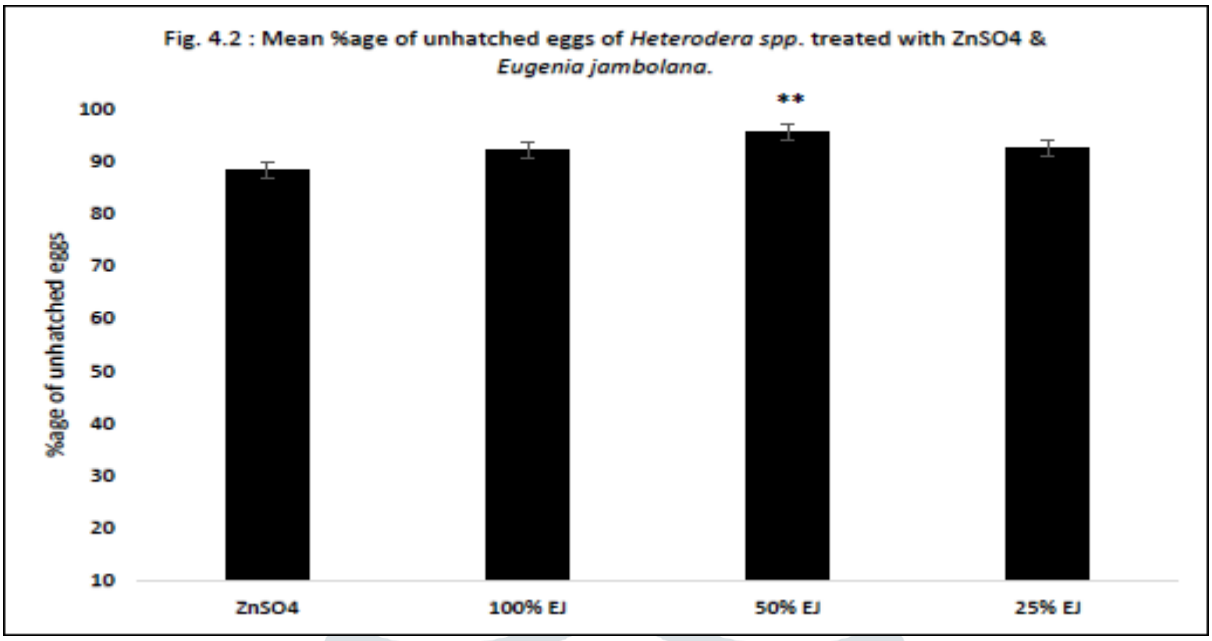
Fig. 3.3 : Mean %age of Comparative Nematostatic effect of *Ficus bengalensis* & *Eugenia jambolana* on *Heterodera spp.*



(** is significant at p< 0.05)

Fig. 4.1: Mean %age of unhatched eggs of *Heterodera spp.* treated with ZnSO4 &*Ficus bengalensis.*





(** is significant at p< 0.05)