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Efficacy of Leaf Extracts of Neem (*Azadirachta indica*) and Chinaberry (*Melia azedrach*) Against Early Blight and Wilt Diseases of Tomato

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Abstract: The efficacy of leaf extracts of neem (*Azadiracta indica*) and chinaberry (*Melia azedarach*) against two tomato pathogenic fungi *Alternaria solani* and *Fusarium oxysporum,* the causal agents of early blight and wilt diseases of tomato plants respectively were studied. A comparison between the *in vitro* activity of neem and chinaberry leaf extracts against these two pathogenic fungi were investigated. Leaf extracts of different concentrations (5%, 10%, 15% and 20 %) of aqueous, ethanol and ethyl acetate of neem and chinaberry were added to growth media prior to inoculation. Inhibition values recorded a characteristic variation between neem and chinaberry. In case of neem, inhibition percentages were 17.88%, 23.66, 52.77 % and 70.55% for *Alt. solani* in the four used concentrations, while those for *F. oxysporum* were 14.77 %, 23.88%, 31.22 % and 100%, respectively. The corresponding values with chinaberry leave extracts were 3.11 %, 5.22%, 5.53 % and 5.77 %, recorded for *Alt. solani* and 5.44 %, 6.11 %, 6.35 % and 6.55% for *F. oxysporum*. Both ethanol and ethyl acetate extracts of neem leaves assayed at a concentration of 20%, completely suppressed the growth of *F. oxysporum* and inhibited *Alt. solani* by ratios between 52.44% and 62.77%, the same extracts but from chinaberry (20%) slightly inhibited the growth of both pathogenic fungi and values of inhibition not exceeded the 7 %. All used concentrations of neem extract effectively suppressed the mycelial growth of both pathogenic fungi and this effect gradually increased with increasing concentration. In addition, tests on activity of the main component, nimonol purified by HPLC as a separate component from neem ethyl acetate extract (mother extract), it gave no inhibition activities at all concentrations. Pathogenicity tests *in vivo* by *Alt. solani* showed disease incidence of 53.20 and 100 % after 2 and 4 weeks. Spraying tomato plants with 20% aqueous neem leaf extracts lowered the disease incidence to 42.54% while spray and irrigation reduced disease incidence to 39.49%. Spray and irrigation with 20% aqueous extracts from neem leaves seemed to work in a synergism in controlling the disease. Disease severity and control of tomato plants were also studied after 2 and 4 weeks of pathogen inoculation, results indicated that higher control ratios were also obtained in plants sprayed and irrigated with the aqueous extracts and values recorded were15.36% and 43.71% after 2 and 4 weeks, respectively with an increase in the disease severity for all treated plants by the progress of inoculation time. Concerning *F. oxysporum*, the effect of (20%) aqueous neem leaf extracts on seed germination, disease incidence and disease control of tomato plants were studied, results showed that the highest percentage (100%) of seed germination was obtained with –ve control (seeds + water) and seeds irrigated with neem leaf extracts, while the lowest one (70%) was recorded with +ve control (seeds + water + pathogen). Regarding disease incidence, the highest value (94.29%) was recorded with +ve control while the lowest (19.04%) was recorded with seeds treated with pathogen and irrigated with neem aqueous extracts whereas the highest disease control (80.96%) was recorded for seeds treated with pathogen and irrigated with neem extract. Neem extracts which are cheap and environmentally safe, exhibited considerable control of disease development and may be considered promising for protection of tomato plants against early blight and wilt pathogens with possible improvement of this economic crop.

Key words: Neem (Azadirachta indica), Chinaberry (Melia azedrach), Aqueous and organic extracts, Antifungal activity, Alternaria solani, Fusarium oxysporum, Tomato, Disease control.

INTRODUCTION

Tomato (*lycopersicon esculentum* Mill) is considered one of the most important and solanaceous vegetable crop in Egypt for local consumption and exportation purposes. Tomato plants are subjected to attack by

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numerous diseases wherever the crop is planted, fungal pathogens, like *Alternaria solani*, the causal agent of early blight disease and *Fusarium oxysporum* f.sp. *lycopersici*, the causal agent of wilt disease are considered as agents of reduction of the crop (Awad, 1990 and Stone *et al*., 2000). Early blight is a three-phase disease, which produce leaf spots, stem canker and fruit rot, but the foliar phase is the most common and destructive part of the disease (Maiero and Barksdale 1989), responsible for significant economic losses sustained by tomato producer each year. *Alt. solani* can cause extensive defoliation leading to a reduction of economic fruit yield (Spletzer and Enyedi, 1999). *Fusarium* wilt disease caused by *F. oxysporum* f.sp. *lycopersici* is one of the most devastating diseases of tomato (Singh *et al.,* 1980). Control of early blight disease has been accomplished primarily by the application of chemical fungicides, long crop rotations, pasteurizing seedbeds with steam or fumigants (Spletzer and Enyedi, 1999). Perusal of earlier literatures indicates that attention has not been given for utilization of plant extracts in controlling *F. oxysporum* and other plant pathogens, even if their effectiveness has been reported in reducing many diseases of various plants (Bansal, and Rajesh 2000). It is therefore necessary to search for control measures that are cheap, ecologically sound and environmentally safe to eliminate or reduce the incidence of economic important pathogens and to increase both seed germination and yield of plant crops. In recent years much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens (Nwachukwu and Umechurub, 2001), medicinal plants are part and parcel of human society to combat disease. Neem (*Azadirecta indica* A. Juss) and chinaberry (*Melia azedarach* L.) are two trees belonging to the mahogany family (Meliaceae), neem is an evergreen tree (Schmutterer, 1995) and it was introduced to Egypt from Sudan around 1963 (Awad, 1990). On the other hand, chinberry is a deciduous tree native to southern Asia Australia (Fish 1989 and Eeware, 1998) and it is an old tree in Egypt (Awad, 1990) with reputed value for is antifungal properties (Bina *et al*., 2004).

Singh *et al*., (1993) reported that treatment of infested banana fruit with aqueous leaf extract of *A. indica* gave good control of *F. oxysporum* disease development with minimum percentage loss in fruit weight and was showed to be among the most effective medicinal plants used. Gamguly, (1994) reported that aqueous neem leaf extract inhibited mycelial growth and spore germination of *Helminthosporium oryzae* and *pyricularia oryzae* responsible for blast and brown spot of rice plant respectively. Srivastava *et al.,* (1997) showed the fungicidal properties of aqueous leaf extracts of *A. indica* against *Alt. alternate* from pear fruits with 85 % control of fruit rot *in vivo*. Lovang and Wildt-Persson (1998) reported that *M. azedarach* aqueous leaf extract was a good inhibitor of *Bipolaris micropus* but it was partially inhibitor to *Alt. solani* with little or no effect on *F. oxysporum* as test pathogens of tomato. Patil *et al*., (2001) showed that neem leaf extract was effective in reducing early blight incidence with increased yield of tomato infected by *Alt. solani*.

Plant extracts have played significant role in the inhibition of seed-borne pathogen *F. oxysporum* and in improvement of seed quality and emergence of plant seeds. (Nwachukwu and Umechuruba, 2001). Ethanol extracts of *A. indica* showed fungal toxic properties against *Alternaria brassicola* and *F. oxysporum* (Chivpuri *et al*., 1997). Kishore *et al*. (2001) reported that ethanol leaf extract of *A. indica* was highly inhibitory to *Phaeoisariopsis personate*, the causal agent of late leaf spot of ground nut. Aboellil (2007) reported that Trilogy, a natural product from *A. indica* was significantly retarded several growth parameters of cucumber powdery mildew pathogen (*Podosphaera xanthii*), and induced resistance in cucumber plants. The objective of this study was to determine the efficacy of aqueous, ethanol and ethylacetate leaf extracts of neem and chinaberry for controlling two important tomato pathogenic fungi, *Alt. solani* and *F. oxysporum*.

MATERIALS AND METHODS

Neem and Chinaberry Leaves:

Neem (*Azadirachta indica* A. Juss) leaves from 8-years-old trees and chinaberry (*Melia azedarach* L.) from 10- years-old trees were obtained from Kalyoubeia governorate, Kalyoub city, Egypt.

Preparation of Aqueous Extracts of Neem and Chinaberry Leaves:

Neem and chinaberry leaves were obtained and utilized for the experiments according to the method described by Shetty *et al.,* 1989 and Achimu and Schlosser, 1992.

Preparation of Organic Extracts of Neem and Chinaberry Leaves:

Organic extracts of neem and chinaberry were prepared according to the method described by

Pankajalakshmi and Taralakshimi, 1994. In this study different types of organic solvents were tested other than ethyl acetate and ethanol, examples include chloroform and methylene chloride.

Nimonol Purification:

Pure nimonol used in this study was obtained by HPLC purification of ethyl acetate extract from neem leaves using high density reversed phase C18 column with elution carried out in a stepwise gradient over 2 hours using HPLC grade solvents (methanol and water).

In Vitro* Experiments *Plant Pathogens:

Alternaria solani and *Fusarium oxysporum* f. sp. *lycopersici,* were obtained from Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Antifungal Effects of Aqueous and Organic Leaf Extracts of Neem and Chinaberry on Growth of Tomato Pathogens, Alternaria Solani and Fusarium Oxysporum:

Measurement of radial growth of the two studied tomato pathogens were taken following the technique used by Dixit *et al.,*1976.

In Vivo Experiments Tomato Plants:

Tomato (*Lycopersicon esculentum* Mill) seeds and seedlings cv *Castle Rock* aged four weeks aged and seeds of the same type were obtained from Agricultural Research Center, Giza, Egypt.

Pathogenicity Test of Alternaria Solani:

This experiment was carried out under greenhouse conditions to evaluate the potential capabilities of the tested fungi according to the method described by Hilaal, 1992. The percentage of infection was determine as usual meantime, the disease severity was estimated after 2 and 4 weeks of inoculation as mentioned by Horsfall and Barratt, 1945.

Pathogenicity Test of Fusarium Oxysporum Inoculum Preparation and Soil Infestation:

The fungus was prepared for inoculation as mentioned by Wong *et al.,* 1984.*F. oxysporum* inoculum (1% weight of maize seeds-based inoculum / weight of steam-pasteurized soil) was thoroughly dispersed through the steamed soil distributed among two sets of pots (3 Kg clay soil each). The first set contained the pathogen and the second set received the same quantities of subsequently autoclaved maize seeds to serve as control. All pots were watered and left for one week to insure even distribution of the inoculum.

Preparation of Seeds for Cultivation:

Tomato seeds were surface sterilized by immersing in 70 % ethanol for 2 min. then in 0.2 % sodium hypochlorite (NaoCl) for 3 min. They were washed for several times with sterile distilled water. Ten seeds / pot were placed into 30 cm diameter pots, each filled with about 3 kg soil. The seeds were sown at 3 cm depth in each pot and when emergence was complete (~10days) the seedling density was reduced to 5 seedlings / pot. The treatment was replicated 5 times with 5 plants /pot. The pots were placed in greenhouse and the free draining pots were watered every other day with equal amount of water. To avoid the nutrient deficiency effect, the plants were irrigated every week with 10 ml of 1/7-strength Hoagland solution (Hoagland, 1944). The growing tomato plants were observed daily for two months and foliar symptom 4 weeks after inoculation.

Determination of Effect of Aqueous Neem Leaf Extract on Early Blight Disease of Tomato:

A concentration of (20%) aqueous neem leaf extract was used since it gave the highest reduction of Alt. solani growth *in vitro*. The test fungus was grown as mentioned before and the inoculum concentration was adjusted at (10^6) using hemicytometer slide. Forty five days age tomato seedlings were subjected to the following treatments and five replicate pots were used for each treatment according to method described by Datar and Mayee, 1985. The inoculated plants were covered with plastic bags for 48 hours as usual to maintain high relative humidity for fungal infection according to the method described by Hilaal (1992). The percentage of infection was determined as usual meantime, the disease severity was estimated after 2 and 4 weeks of inoculation as mentioned by Horsfall and Barratt, 1945.

Determination of Effect of Aqueous Neem Leaf Extract on Wilt Disease of Tomato:

This experiment was carried out under greenhouse condition to evaluate the effect of aqueous neem leaf extract on tomato plants according to the method applied by Wong *et al*., 1984.

Preparation of Seeds for Cultivation:

Surface sterilized tomato seeds were subjected to the treatments described by Ganapathy and Narayanasamy, 1990. Seeds were placed into 30 cm diameter pots (10/pot) each filled with about 3 Kg soil, seeds were sown to a depth of 3 cm in each pot. After seed germination the seedlings were reduced to 5 seedlings / pot and each treatment was replicated 5 times. The free draining pots were watered every other day. Wilt disease was started to appear after four weeks of sowing.

RESULTS AND DISCUSSION

In vitro Experiments:

Effect of Aqueous Extracts from Neem and Chinaberry Leaves on Tomato Pathogens:

All concentrations of aqueous neem leaf extracts suppressed the mycelial growth of the two tested pathogens, the effect was proportional to concentration and inhibition values were higher for extracts of neem than those of chinaberry. The results show that aqueous leaf extracts of neem caused inhibition in growth of the two tested fungi by different ratios. Figure (1) shows a marked effect of the 20% aqueous extracts from neem with inhibition values of 100 and 70.55% for *F. oxysporum* and *Alt. solani* whereas those from chinaberry inhibited the growth of the same pathogens by 6.55 and 5.77%.

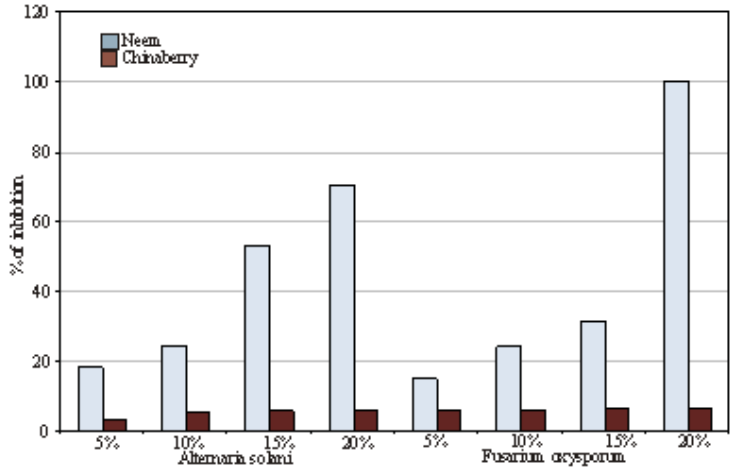

Fig. 1: Effect of different conc. of aqueous extracts from neem and chinaberry on growth of tomato pathogens.

Effect of Ethanol Extracts from Neem and Chinaberry Leaves on Tomato Pathogens:

Extraction was carried out using ethanol and ethyl acetate solvents, and data revealed higher characteristic effects of neem than chinaberry, the ethanol neem leaf extract effectively suppressed the mycelial growth of the two pathogenic fungi and this effect was increasing gradually with concentrations and a concentration of (20%) completely suppressed the growth of *F. oxysporum*. Figure (2) illustrates the inhibition in growth of the two tested fungi using ethanol extracts of neem and chinaberry leaves, *F. oxysporum* was inhibited by 100% using 20% concentration of extract and 52.44% with *Alt. solani*, those of chinaberry were 6.68% and 6.15% for both pathogens, respectively.

Effect of Ethyl Acetate Leaf Extracts from Neem and Chinaberry on Tomato Pathogens:

Results indicate that inhibition values of ethyl acetate extracts from the neem leaf were extremely higher in comparison to those of chinaberry, and used concentrations effectively suppressed the mycelial growth of the tested fungi and this effect gradually increased with concentration, only the concentration of (20%) was able to completely prevent the mycelial growth of *F. oxysporum*. Results are presented in Figure (3), the effect of (15%) ethyl acetate extracts from neem and chinaberry leaves on the growth of the two pathogenic fungi could be noticed and for that of neem, a characteristic inhibition in growth of *Alt. solani* (62.33%) and of *F. oxysporum* (60.44%) was achieved. When assay was carried out with a higher concentration (20%), the data revealed a stronger inhibition activity of ethyl acetate extract on both.

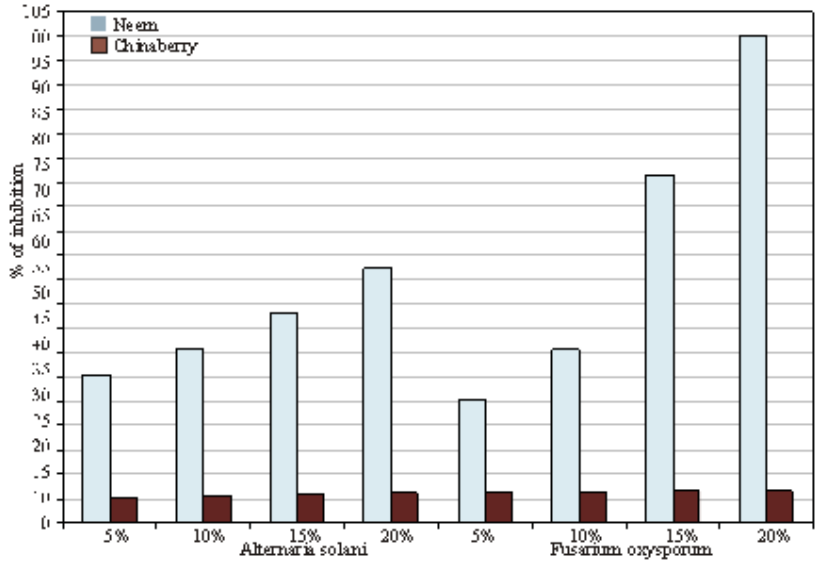

Fig. 2: Effect of different conc. of ethanol extracts from neem and chinaberry on growth of tomato pathogens.

Effect of Pure Nimonol from Neem Ethyl Acetate Extract on Tomato Pathogens:

Results showed that purified nimonol have no antifungal activities against the two test pathogens at any concentration and when testing the active mother ethyl acetate extract free of nimonol (20%), inhibition values were only 10.46% in case of *Alt. solani* and 13.36 for *F. oxysporum* but when nimonol was re-added to the mother organic extract, values of inhibition raised to 35.48 % and 42.12% (Figure 3).

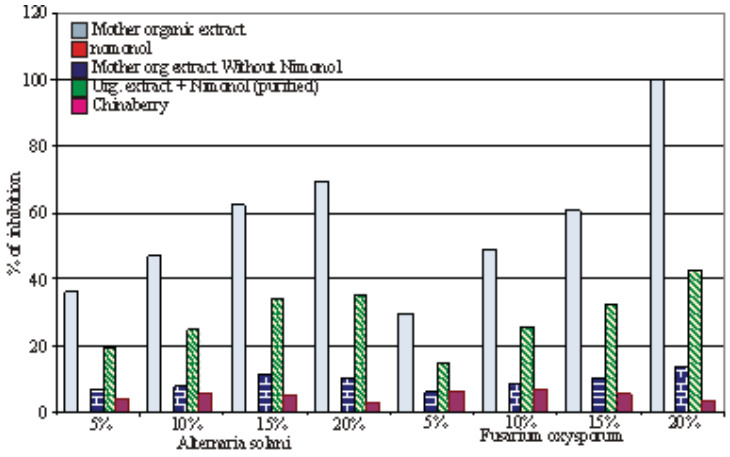

Fig. 3: Effect of different conc. of ethyl acetate extract and nimonol in comparison to inaberry on growth of tomato pathogens.

In Vivo Experiments Pathogenicity Tests of Alternaria Solani:

Symptoms of early blight disease were first observed as small, black lesions mostly on the older foliage leaves after 2 and 4 weeks of inoculation. Spots enlarged by the time and they were one-fourth inch in diameter or larger, concentric rings in a bull's eye pattern could be seen in the center of the diseased area. Tissue surrounding the spots turned yellow as a result of high temperature and humidity and caused killing of the foliage. Results showed that the percentage of disease incidence was 53.20 % after 2 weeks and reached 100 % after 4 weeks of inoculation.

Effect of Aqueous Neem Leaf Extracts (20%) on Disease Incidence and Control of Tomato Early Blight after 2 and 4 Weeks of Inoculation:

Figure (4) is showing that after 2 weeks of inoculation by *Alt. solani*, the disease incidence was 53.20 % for +ve control and it was lowered to 42.54%, when tomato plants were sprayed with 20% aqueous neem leaf extracts. On the other hand, tomato both sprayed and irrigated with the same concentration showed more reduction in disease incidence (39.49 %) and the percentages of control after 2 weeks of inoculation were 10.66 % and 13.71 % for plants sprayed and both sprayed and irrigated with the extracts. Also, results shows that after 4 weeks, the disease incidence were 100.0 %, 79.20 % and 71.67 % for +ve control, plants sprayed and plants both sprayed and irrigated, respectively. The percentages of control after 4 weeks were 20.80 % and 28.33 % for plants sprayed and both sprayed and irrigated with neem leaf extracts, respectively. Figure (5) illustrate the percentage of disease severity in tomato plants after 2 weeks which were 26.81 %, 16.75 % and 11.45 % for +ve control, plants sprayed with 20 % aqueous neem leaf extracts and plants sprayed and irrigated with the same extract concentration. These percentages increased after 4 weeks and were 61.66%, 26.19% and 17.95% for the same sets of plants.

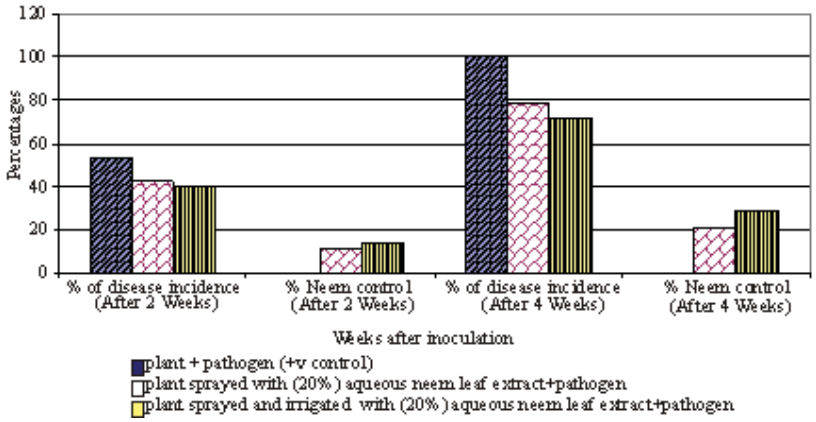

Fig. 4: Effect of 20 % aqueous neem leaf extracts on severity and control of tomato early blight after 2 and 4 weeks of inoculation.

Pathogenicity Tests of Fusarium Oxysporum:

The wilt disease started to appear after 4 weeks, the morphological symptoms appeared in the form of chlorosis, dropping and epinasty of leaves followed by yellowing, stunting and death of plant. The percentage of germinated seeds in fungal infested soil was 70 % compared with 100 % germinated seeds in the control soil, also, the percentage of disease incidence was found to be 94.29 % in infested soil.

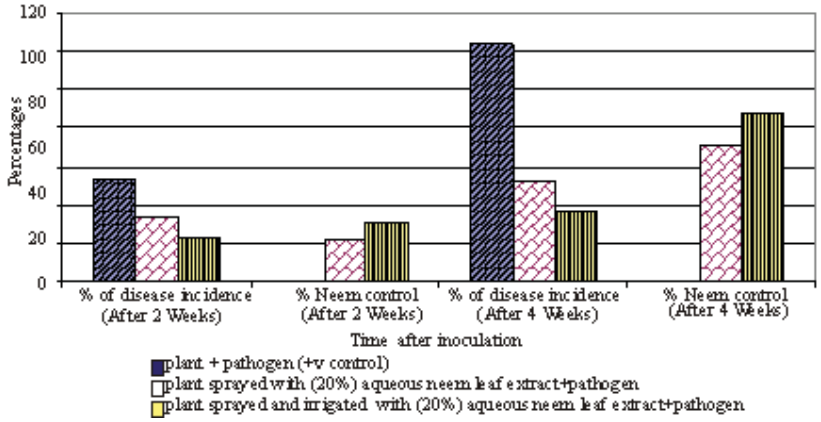

Fig. 5: Effect of 20 % aqueous neem leaf extracts on severity and control of tomato early blight after 2 and 4 weeks of inoculation.

Effect of Aqueous Neem Leaf Extracts (20%) on Seed Germination, Disease Incidence and Control of Tomato Wilt:

The two highest percentages (100%) of seed germination recorded with –ve control and with seeds irrigated with 20 % aqueous neem leaf extracts, are reported in Fig. (6) which shows a lower one (70.0 %) recorded in + ve control. Concerning disease incidence, the results indicated that the highest value (94.29%) was recorded with + ve control, while the lowest (19.04 %) was recorded with seeds treated with pathogen and irrigated with aqueous neem leaf extracts. On the other hand, tomato seeds treated with the pathogen and irrigated with neem extracts showed the highest percentage of disease control (80.96 %) compared with 5.71% for + ve control.

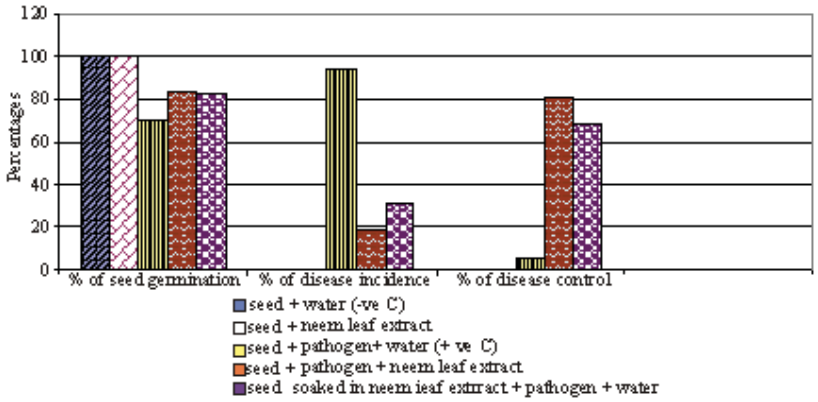

Fig. 6: Effect of aqueous neem leaf extracts (20%) on seed germination, disease incidence and disease control of tomato wilt.

Discussion:

Biological control had attained importance in modern agriculture to curtail the hazards of intensive use of chemicals for pest and disease control (Tuber and Baker, 1988). Accordingly, the efficacy of different plant extracts from leaves of neem and chinaberry against *Alt. solani* and *F. oxysporum*, the causal agents of early blight and wilt diseases of tomato were studied *in vitro* and *in vivo*.

Chinaberry leaf extracts at all concentrations, showed a slightly inhibition for the two selected pathogens thus was excluded from further studies whereas neem aqueous leaf extracts were found to be highly effective in controlling the growth of the two tested pathogens. All types of extracts from neem leaf showed different levels of antimicrobial activity and the relative differences were found to vary within the tested extracts and with increasing concentration of the extracts, a gradual increase in the inhibition potential of the tested fungi was recorded.

In this study, four concentrations of the aqueous neem leaf extracts effectively suppressed mycelial growth of the two pathogens, the 20% concentration completely suppressed the growth of one of them, *F. oxysporum* (100%). These results are in agreement with (Singh *et al*., 1980) who found that the growth of four pathogens (*F. oxysporum*, *R. solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*) which incite wilt and rot in *Cicer arietinum*, was inhibited in liquid medium by extracts of leaf, trunk park and oil from the neem tree. Also, Sivakadadcham, (1988) mentioned that extracts from *A. indica* were inhibitory to *R. solani* but none of the leaf extracts tested was effective against *F. solani* and results indicated that different green manures could selectively suppress or enhance microbial populations in soil. Later, Bankole and Adebanjo (1995) showed that neem leaf extract inhibited the growth of four pathogenic fungi (*Macrophomina phaseolina*, *F. moniliforme*, *F. solani*, and *Botryodiplodia theobromae*) *in vitro*. Meena and Mariappan (1993), also reported the inhibitory effect of neem leaf extract on growth and spore germination of seed mycoflora including *Alternaria tenuis* , *A. flavus*, *Curvularia lunata, F. moniliforme* and *Rhizopus stolonifer*. Different extracts from neem leaves were found to have inhibitory effect on *Rhizoctonia solani* (Sharma and Jnandaik, 1994) and *Trichoderma viride*, *F. oxysporum* and *Pythium aphanideratum* (Sharma, 1998). Dwivedi and Shukla (2000) found that the mycelial growth inhibition rate increased with plant extract concentration, also, 100% aqueous neem (*A. indica*) leaf extract caused complete inhibition of spore germination of *Fusarium* spp.

Comparing leaf extracts of ethyl acetate with ethanol, the inhibition percentage in each increased gradually with the extract concentration, both completely suppressed the growth of *F. oxysporum* but the ethyl acetate one showed higher inhibition on the growth of *Alt. solani*. Results of the present study are in agreement with Shivpuri *et al.,* (1997) who noticed that ethanol extracts of *A. indica* showed fungitoxic properties against 5 pathogenic fungi (*Alternaria brassicola*, *Colletotrichum capsici*, *F. oxysporum*, *R. solani* and *Sclerotinia sclerotiorum*) when tested under laboratory conditions at 500 and 1000 µg/ml. And with notes of Banumathy (1998), who reported that active compounds of neem are distributed throughout the tree parts but are concentrated in seeds and leaves, and were extractable by water and organic solvents and of Govindachari *et al*., (1999) who reported that the hexane extract of neem leaves and its fractions had antifungal activity against two plant pathogens, *F. oxysporum* and *Colletotrichum lindemuthianum* with a correlation of their activity to high concentrations used**.** These findings agree with Upasana *et al.,* (2002) who found that both ether and methanol neem seed extracts gave the best results at 3000 µlitre/100 ml concentrations but the methanol was found effective against *A. niger*, *F. oxysporum* and *Trichoderma resii* whereas, maximum inhibition zone was seen in ether extract only against *F. oxysporum*.

One of ten components, most of them identified by HPLC in the active organic extract (Data not shown), showed no antifungal activity when assayed as a separate component at different concentrations, also the activity of the mother organic extract was significantly lowered after pooling this fraction. This may be explained by the presence of a kind of synergism between the different neem components and which is responsible for the antifungal properties of the extract.

Spraying tomato plants with 20 % aqueous neem leaf extract slightly decreased early blight incidence and disease severity while spray and irrigation with the same extract caused a marked decrease in both disease incidence and severity after two and four weeks of inoculation. Results were in conformity with those by Chattopadhyay (1999) who found that foliar spray of *A. indica* leaf extract and azadirachtin reduced mycelial growth of *Alt. alternata* (causing loss of sunflower and tomato), decreased disease severity and increased yield over control. Babu *et al.,* (2000) mentioned that spraying with 3 % of neem oil in tomato pot cultures resulted in 53 % reduction in disease incidence over the control while Patil *et al.,* (2001), found that incidence of tomato early blight caused by *Alt. solani* was affected by a botanical like neem seed extract with increased fruit yield between 156.43 and 168.56q / ha. Also, similar results were obtained by Amadioha and Uchendu, 2003 who concluded that extracts from neem plant, especially the bark, could be used by farmers to control the rot of tomato fruit caused by *F. solani* during storage and by Chaudhary *et al.,* (2003) who tested *in vitro* different plant extracts including *A. indica* against *Alt. alternata* causing early blight of potato, and proved that bulb extracts of *Allium sativum* caused 59% inhibition followed by extracts of *A. indica* (54%). Recently, Sanjeet *et al.,* (2005) found that extracts of *A. indica* highly controlling leaf spot in faba beans caused by *Alt. alternata* under laboratory and field conditions. The marked reduction in the severity of early blight pathogen when plants were treated with aqueous neem leaf extract may be related as mentioned previously to the toxic antifungal substances present in the aqueous extract and/or to change of soil pH due to the alkaline nature of the neem leaves (National Research Council, 1992). It's important to note that the irrigation of uninfected tomato seeds with 20 % aqueous neem extract (2 L /pot) after 4, 6 and 8 weeks of sowing for both pathogens, highly improved the germination with significant increase in various growth parameters of treated tomato plants especially after the 8th week followed by infected seeds irrigated with the extract (Data not shown). In conclusion, the present study explores the possibilities of controlling *Alt. solani* and *F. oxysporum* by using leaf extracts of neem and highlights on results encouraging the possible application in agriculture after field investigations.

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