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Efficacy of different aqueous plant extracts on the growth of *Alternaria solani,* a foliar pathogen of tomato

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M. Magesh[1], R. Sudha Raja Kumar[1] and P. Ahila devi[2]

[1]Annamalai University Annamalainagar-608 002, Chidambaram, Tamilnadu, India.
[2]Tamil Nadu Rice Research Institute (TNAU), Aduthurai-612 101, Tamilnadu, India.

Abstract

Aqueous plant extracts of *Allium sativum, Azadirachta indica*, *Eucalyptus globules* and *Zingiber officinale. Allium sativum* were tested against the growth of *Alternaria solani* and on the seed germination, shoot length, root length and vigour index of tomato at 15 per cent concentration. There was100 per cent inhibition of the mycelial growth of *A. solani,* and maximum percentage of seed germination (90.00%), shoot length (7.2 cm), root length (9.1cm) and vigour index (1467.2) of tomato seedlings were recorded *in vitro* in the presence of the plant extract of *Allium sativum* , tested following the method of Roll Towel method.

Keywords: Tomato, *Alternaria solani,* plant extract- *Allium sativum*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family Solanaceae is one of the most important cash crops and widely grown vegetable in the world. It is cultivated for its fleshy fruit and the area under its cultivation is increasing day by day due to its nutritional value, demand and high yield. Tomato is a regular kitchen component of Indian diet which is cooked in the form of various processed products like juice, ketchup, sauce, pickle, pastes and powder. It also has high medicinal value; the pulp and juice is digestible, promotes gastric secretion and purifies blood. It is an excellent source of folate, potassium, vitamin A and C as well as lycopene – a natural antioxidant, which is not found in any other solanaceous crops (Anonymous, 2008). It is grown in an area of 22,433 ha, with a production of 2, 82,912 tones with a productivity of 12,611 kg/ha in Tamil Nadu (Anonymous, 2013).

India is the fourth largest producer of tomato globally, contributing around 11.9 MT/year. However, its average per hectare production is lesser (19.6 MT) when compared to the world average (28.2 MT) (Anonymous, 2014). The yield of tomato is restricted to a great extent due to various diseases and insect pests associated with tomato cultivation. Amongst these, tomato is highly susceptible to early blight, late blight and *Fusarium* wilt (Panthee and Chen, 2010). Fungal infestations cause deterioration in the quality of tomato, minimize yield and fetch less market value.

*Corresponding Author :
email: *ahila.devi1@gmail.com*

Moreover, there is an increased public demand for sustainable and chemical residue-free food production (Arthur, 1996). In response to this, biofungicides, derived either from microbes or plants, emerged as promising alternative strategies. Neem (*Azadirachta indica*), garlic (*Allium sativum*), onion (*Allium cepa)* and few other plants inhibit early blight in potato and tomato (Prasad and Naik, 2003; Mate *et al*., 2005). Leaf extracts of *Prosopis juliflora* and *Cocos nucifera* inhibited proliferation of spores of *Alternaria. solani* above 90% (Thiribhuvanamala *et al*., 2001). Plants provide abundant resource of antimicrobial compounds and have been used for centuries to inhibit microbial growth (Jun-Dong *et al.,* 2006). The extracts of many allelopathic plants are now known to exhibit antimicrobial activities. Flavanoids, triterpenoids, steroids and other phenolic compounds in plants have been reported to have antimicrobial activity (Hostetman *et al.,* 1995). The potential biological activity of plant extracts had been assessed against a wide range of fungal phytopathogens. The present article deals with the determination of effective plant products /extract against *A. solani* under *in vitro* and *in vivo* conditions, and assess of the effect of plant extracts on the biometrics of tomato and early blight incidence.

MATERIALS AND METHODS

Testing the *in vitro* efficacy of plant extracts against *A.solani*

Food poisoning technique

Potato dextrose agar (PDA) medium was mixed separately with aqueous extracts of four different plant species at different concentrations *viz*., 5%, 10%, 15% and 20% and poured into sterile Petri dishes, allowed

to cool and solidify. Mycelial disc (5 mm) of seven days old culture of *A. solani* was placed at the centre of each Petri dishe and incubated at 28±2 °C for 7 days, The PDA medium without plant extracts but with the same concentration of sterile distilled water served as control. Similarly a fungicide *viz*., mancozeb (0.25% conc.) was also tested against the pathogen for comparison. Three replicates were maintained. The per cent inhibition of the mycelial growth, if any, was determined by the formula

$$PI = C - T / C \times 100$$

(where C = Diameter of *A. solani* in control, T = Diameter *A. solani* in treatment)

Table 1. Evaluation of plant extracts against *A. solani* under *in vitro* condition by agar well method.

S.No	Treatment	Mycelia growth (mm)				Per cent inhibition over control			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Allium sativum*	23.42^{b}	19.95^{b}	17.44^{b}	15.98^{b}	73.97^{b}	77.83^{b}	80.62^{b}	82.24^{b}
2.	*Azadirachta indica*	42.76^{e}	41.34^{e}	40.74^{e}	38.54^{e}	52.48^{e}	54.06^{e}	54.73^{e}	57.17^{e}
3.	*Eucalyptus globules*	31.64^{d}	33.17^{d}	34.38^{d}	36.41^{d}	64.84^{d}	63.14^{d}	61.80^{d}	59.54^{d}
4.	*Zingiber officinalis*	28.36^{c}	26.47^{c}	25.18^{c}	24.74^{c}	68.48^{c}	70.58^{c}	72.02^{c}	72.51^{c}
5.	Mancozeb 75% WP (0.25% conc.)	7.27^{a}				91.92$_{a}$			
6.	Control	90^{f}				0^{f}			

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 2. *In vitro* evaluation of different plant extracts at different concentrations against *A. solani* (Poisoned food technique).

S.No	Treatment	Mycelia growth (mm)				Per cent inhibition over control			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Allium sativum*	15.02^{b}	10.13^{b}	3.05^{b}	0.0^{a}	83.31^{b}	88.74^{b}	96.61^{b}	100^{a}
2.	*Azadirachta indica*	25.21^{e}	23.74^{e}	20.18^{e}	17.21^{d}	71.98^{e}	73.62^{e}	77.57^{e}	80.87^{b}
3.	*Eucalyptus globules*	21.76^{d}	17.32^{d}	15.34^{d}	14.06^{c}	75.82^{d}	80.75^{d}	82.95^{d}	84.37^{c}
4.	*Zingiber officinalis*	19.42^{c}	15.29^{c}	10.97^{c}	8.63^{b}	78.42^{c}	83.01^{c}	87.81^{c}	90.41^{b}
5.	Mancozeb 75% WP (0.25% conc.)	0^{a}	0^{a}	0^{a}	0^{a}	100^{a}	100^{a}	100^{a}	100^{a}
6.	Control	90^{f}	90^{f}	90^{f}	90^{e}	0^{f}	0^{f}	0^{f}	0^{f}

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 3. Effect of plant products on the mycelial dry weight of *A.solani* (liquid medium).

S.No	Treatment	Mycelial dry weight (mg)				Per cent inhibition			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Allium sativum*	0.175^{b}	0.156^{b}	0.00^{a}	0.00^{a}	99.80^{b}	99.82^{b}	100^{a}	100^{a}
2.	*Azadirachta indica*	0.212^{e}	0.205^{e}	0.198^{d}	0.174^{d}	99.76^{e}	99.77^{e}	99.78^{d}	99.80^{d}
3.	*Eucalyptus globules*	0.194^{d}	0.185^{d}	0.171^{c}	0.167^{c}	99.78^{d}	99.79^{d}	99.81^{c}	99.81^{c}
4.	*Zingiber officinalis*	0.189^{c}	0.175^{c}	0.158^{b}	0.135^{b}	99.79^{c}	99.80^{c}	99.82^{b}	99.85^{b}
5.	Mancozeb 75% WP (0.25% conc.)	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	100^{a}	100^{a}	100^{a}	100^{a}
6.	Control	3.00f				96.66f			

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 4. Effect of plant extracts on the seed germination and growth parameters of tomato seedling.

S.No	Treatment sources	Concentration (%)	Shoot length (cm)	Root length (cm)	Seed germination (%)	Vigour index
1.	*Allium sativum*	15	7.2[a]	9.1[ab]	90.0[a]	1467.00[b]
2.	*Azadirachta indica*	15	5.2[c]	6.0[d]	80.0[d]	896 .00[e]
3.	*Eucalyptus globules*	15	5.0[c]	8.6b[c]	84.2[c]	1145.12[c]
4.	*Zingiber officinale*	15	6.5[b]	6.3[d]	88.5[b]	1132.8[d]
5.	Mancozeb 75% WP	0.25	7.4[a]	9.8[a]	91.0[a]	1565.2[a]
6.	Control		4.5[d]	7.5[c]	74.5[e]	894.00[e]

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Dry mycelial weight

Potato dextrose PD broth was mixed separately with extracts of four different plant extracts at different concentrations *viz*., 5%, 10%, 15% and 20%. The concentration chosen for each of the test fungus was based on the Minimum Inhibitory Concentration (MIC) value obtained from food poisoning technique. Mycelial discs (5 mm) of seven days old culture of *A. solani* were inoculated in PD broth. The flask containing the respective medium without the plant extract but with equal volume of sterile distilled water served as control. For each treatment three replicates were maintained. All the inoculated flasks were incubated at 25±2 °C for 15 days. After the incubation period, the contents of each of the flask were filtered through a pre weighed Whatman No 1. filter paper. The mycelial dry weight was determined by subtracting the weight of the filter paper from the total weight.

Agar well diffusion method (Thongson *et al*., 2004)

Spore suspension of the fungal culture was prepared with sterile distilled water from 7 days old culture. Desired concentrations of the selected plant extracts were prepared. 20 ml of PDA medium was seeded with 3 ml of spore suspension (1×10^6 spore / ml) and allowed to solidify. Wells were cut equidistantly with the aid of the sterile cork borer. Test plant extracts (100µl) of different concentrations were pipetted out separately and poured into each well. Mancozeb @ 0.25% conc. was used for comparison. Suitable control was maintained for each treatment. The plates were incubated at 28± 2 °C and the inhibition zone of the fungal growth around each well was recorded.

Roll towel method (ISTA, 1993)

The germination paper was soaked in water for 2-4 hr to moist it evenly and to remove any water soluble toxic substances, if present. The seeds treated with the selected plant extracts at different concentrations were placed separately between the two sheets of paper towel (27×20 cm) equidistantly, rolled carefully ensuring no pressure on the seed, wrapped with polythene sheet to reduce surface evaporation and kept in germination chamber in an upright position and incubated at room temperature at 28±2 °C for fifteen days. Each treatment was replicated thrice. Observations were made on the germination percentage, shoot length and root length using the standard procedures after the incubation period was over.

The germination percentage was calculated by using the formula,

$$\text{Germination (\%)} = \frac{\text{number of seeds germinated}}{\text{total number of seeds sown}} \times 100$$

The vigour index was calculated by using the formula (Abdul Baki and Anderson, 1973).

RESULTS AND DISCUSSION

Evaluation of different plant extracts against *A. solani*

In the present study the plant extracts showed varying degree of growth inhibition against *A. solani*. The inhibition of growth of fungus increased with increase in the concentration of the aqueous extract of the test plants. Among the plant extracts tested against *A.solani, Allium sativum* at 15% conc. showed significantly the highest reduction in the radial growth, and biomass production.

In *Allium* plants, different biological active compounds such as, alliin, allicin, allicipin, saponins, steroids, flavones, fistulosin and polyphenol carboxylic acids, ajoene have been reported (Singh *et al.,* 1990b; Carotenuto *et al.,* 1999; Phay *et al*., 1999; Barile *et al.,* 2007; Huma *et al.,* 2009; Parvu *et al.,* 2010). Singh *et al.* (1990) reported that Ajoene a compound derived from garlic inhibited spore germination of some fungi including *Alternaria solani*, *Alternaria tenuissima*, *Alternaria triticina,* and *Fusarium* spp. which cause serious diseases in many important crop plants in India. Bagiu *et al*. (2012) showed that allicin and S-methyl cystein in *A. ursinum* were observed to be

responsible for antimicrobial activity. Besides, it was reported that the antifungal activity of flower extract of this plant species was stronger than leaf extract. This was because of higher allicin content in flower part of the plant (Parvu *et al*., 2011). Parvu *et al.* (2009) stated that allicin is an important antifungal active compound in hydro alcoholic extract of *A. obliquum*. The quality and quantity of the antimicrobial substances in *Allium* sp. depend on plant part used, plant species, harvest time and geographical conditions. Therefore, the antifungal activity observed in this study might be due to the presence of allicin, aliin, saponin, S-methyl cystein or a combination of all of them in *A. hirtifolium.*

In the present study leaf extract of *Allium sativum* at 15 per cent concentration as foliar spray (30 and 45 DAT) recorded reduction in the early blight incidence. Recently the control of pest and diseases with the use of botanical derivatives is gaining more importance. Some compounds of plant origin have proved their effectiveness as fungitoxicants (Dixit *et al*., 1978). Many plant species were reported to possess substances which may either act on the pathogen or to induce resistance in host plant resulting in considerable reduction of plant diseases (Datar, 1988). Biological active compounds which are present in plants act as elicitors to induce resistance in host plants resulting in a reduction of plant disease development (Vidhyasekaran, 1992). The presence of antimicrobial compound (alliin, allicin, allicipin, saponins, steroids, flavones, fistulosin and polyphenol carboxylic acids, ajoene) in the extract of *Allium sativum* could have inhibited the pathogen and also induced resistance in host plants resulting in considerable reduction of early blight incidence observed in the present study.

Efficacy of plant extracts *viz*., *Cinnamomum Zeylanicum* (Bowers and Locke, 2004) *Vitex negundo* (Panda *et al.,* 2009), *Psoralea corylifolia* (Gidwani *et al*., 2010), *Boswellia serrata* (Raja *et al*., 2011), *Calendula officinalis* (Bissa and Bora, 2011) for the control of *A. solani* have been reported. These earlier reports are in line and corroborates with the present study.

REFERENCES

Abudul Baki, A. A. and Anderson, J.D. 1973. Vigour determination in soyabeen seed by multiple criteria. *Crop Science,* 13: 630-633. https://doi.org/10.2135/cropsci1973.0011183X001300060013x

Anonymous. 2008. Ministry of agriculture and Land Reclamation. *Year Book of Department of Agricultural Economic Statistical*. P. 83.

Anonymous. 2013. Indian Horticulture Database. National Horticulture Board, Department of Agriculture Cooperation, Ministry of Agriculture.

Anonymous. 2014. 2000-2011tomato statistics EN.aspx. www.novagrim.com

Arthur, F. H. 1996. Grain protectants: current status and prospects for the future, *J of Stored Products Research*. 32: 293 - 302. https://doi.org/10.1016/S0022-474X(96)00033-1

Bagiu, R.V., Vlaicu, B. and Butnariu, M. 2012. Chemical composition and *in vitro* antifungal activity screening of the *Allium ursinum* L. (Liliaceae). *Int. J. Mol. Sci.* 13: 1426-1436.PMid:22408399 PMCid:PMC3291968 https://doi.org/10.3390/ijms13021426

Barile, E., Bonanomi, G., Antignani, V., Zolfaghari, B., Sajjadi, S.E., Scala, F. and Lanzotti, V. 2007. Saponins from *Allium minutiflorum* with antifungal activity. *Phytochemistry*. 68: 596-603.PMid:17118413 https://doi.org/10.1016/j.phytochem.2006.10.009

Bissa, S. and Bohra, A. 2011. Antibacterial potential of pot marigold. *J. of Microbiology and Antimicrobials.* 3: 51 - 54.

Bowers, H. J. and Locke, J. C. 2004. Effect of formulated plant extracts and oil on population density of *Phytophthora* blight in the greenhouse, *Plant Diseases.* 88: 11 - 16. PMid:30812449 https://doi.org/10.1094/PDIS.2004.88.1.11

Carotenuto, A., Fattorusso, E., Lanzotti, V. and Magno, S. 1999. Spirostanol saponins of *Allium porrum* L. *Phytochemistry*. 51: 1077-1082. https://doi.org/10.1016/S0031-9422(98)00712-2

Datar, V.V. 1988. Antifungal activity of plant extracts against *Fusarium* sp. causing rhizome rot of ginger. Abstracts of papers presented in National Seminar on management of Crop Diseases with plant products/ biological agents. Agricultural College and Research Institute Madurai, India. P.28.

Dixit, V. P., Sinha, R. and Tank, R. 1978. Effect of Neem seed oil on the blood glucose concentration of normal and alloxan diabetic rats. *J. Ethnopharmacol*., 17(1):95-108. https://doi.org/10.1016/0378-8741(86)90076-0

Gidwani, B., Alaspure, R. N. and Duragkar, N. J. 2010. Anti-inflammatory and anti-microbial activity of hexane extract of seed of *Psoralea corylifolia* Linn., *International Journal of Pharmaceutical Research and Development,* 2:129 - 137.

Hostetman, A., Marston, M., Maillard, M. and Hamburger, M. 1995. Proceedings of the phytochemicals society of Europe. *Phytochemistry of plants used in traditional medicine*. P. 17-43.

Huma, Z., Vian M.A., Maingonnat. J.F. and Chemat. F. 2009. Clean recovery of antioxidant flavonoids from onions: Optimizing solvent free microwave extraction method. *J. Chromatogr.* 1216: 7700-7707. PMid:19800069 https://doi.org/10.1016/j.chroma.2009.09.044

ISTA. 1993. Proceedings of International Seed Test Association, International rules for seed testing. *Seed Science and Technology*. 21: 1–152.

Jun-Dong, Z., Zheng, Xu., Young- Bing, C., Hai-Sheng, C., Lan, Y., Mao-mao, A., Ping-Hui, G., Yan, W., Xin-Ming, J. and Yuan-Ying, J. 2006. Antifungal activities and action mechanisms of compounds form *Tribulus terrestris*.L. J. *Enthanopharmacol*. 103:76-84. PMid:16169173 https://doi.org/10.1016/j.jep.2005.07.006

Mate, G. D., Deshmukh, V. V., Jiotode, D. J., Chore, N. S. and Dikkar, M. 2005. Efficacy of plant products and fungicides on tomato earlyblight caused by *Alternaria solani*, *Research on Crops,* 6: 349 - 351.

Panda, S. K., Thatoi, H. N. and Dutta, S. K. 2009. Anti-bacterial activity and phytochemical screening of leaf and bark

extracts of *Vitex negundo* I. from similipal biosphere reserve, *Journal of Medicinal Plant Research*, 3: 294 - 300.

Panthee, D. R. and Chen, F. 2010. Genomics of fungal resistance in tomato, *Current Genomics,* 11: 30 - 39. PMid:20808521 PMCid:PMC2851114 https://doi.org/10.2174/138920210790217927

Parvu, M., Parvu, A.E., Rosca-Casian and O. Parvu. 2010. Antifungal properties of *Allium ursinum* L., ethanol extract. *J. Med. Plants Res.,* 5: 2041-2046.

Parvu, M., Parvu, A.E., Rosca-Casian and O. Parvu. 2011. Antifungal properties of *Allium ursinum* L., ethanol extract. *J. Med. Plants Res.,* 5: 2041-2046.

Parvu, M., Parvu, A.E., Rosca-Casian, O., Vlase, L. and G. Groza. 2009. Antifungal activity of *Allium obliquum*. *J. Med. Plants Res.,* 4: 138-141.

Phay, N., T. Higashiyama, M. Tsuji, H. Matsuura, Y. Fukushi, A. Yokota and F. Tomita. 1999. An antifungal compound from roots of Welsh onion. *Phytochemistry*, 52: 271-274. https://doi.org/10.1016/S0031-9422(99)00170-3

Prasad, Y. and Naik, M.K. 2003. Evaluation of genotype fungicides and plant extracts against early blight of tomato caused by *Alternaria solani*. *Indian Journal of Plant Protection,* 30 (2): 49-53.

Raja, A. P., Furgan, A., Khan, I. A., Shaw, A. S., Aror, D. S., Shah, B. A. and Taneja S. C. 2011. Anti-staphylococcal and biofilm inhibitory activities of acetyl 11- keto â – boswellic acid from *Boswellia serrata*, *BMC Microbiology*, 11:54 - 62. PMid:21406118 PMCid:PMC3066120 https://doi.org/10.1186/1471-2180-11-54

Singh, U.P., V.N. Pandey, K.G. Wagner and K.P. Singh. 1990. Antifungal activity of ajoene, a constituent of garlic (*Allium sativum*). *Can. J. Bot.*, 68: 1354-1356. https://doi.org/10.1139/b90-172

Thiribhuvanamala, G., Ramkrishnan, G. And Sabitha, D. 2001. Effect of plant products against *Alternaria solani*, the early blight pathogen of tomato, *South Indian Horticulture,* 49: 257 - 260.

Thongson, C. P. M. Davidson, W. Maha karchanakul. J. Weijs., 2004. Antimicrobial activity of ultrasound assisted solvent – extracted spices. *Letters Appl. Microbuial.,* 39: 401-404. PMid:15482429 https://doi.org/10.1111/j.1472-765X.2004.01605.x

Vidhyasekaran, P. 1992. Principles of Plant Pathology. New Delhi, CBS printer and publishers. P.8-12.