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Management of Onion Leaf Blight by *Alternaria alternata* (FR.) Keissler by Botanicals and Bio-control Agents

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Abstract: Field experiment was carried out to assess the efficacy of plant oils, plant extracts and antagonistic microorganisms against leaf blight disease of onion caused by *Alternaria alternata*. Two sprays of neem oil (3%) given on onion plants at the first appearance of the disease and the second on 15 days later recorded significantly the lowest percent disease index (22.22%) and besides increasing the yield. In plant extracts *Acorus calamus* rhizome extract 10% was significantly reduced the disease incidence (34.78%) followed by *Mentha arvensis* leaf extract.

Key words: Onion, *Alternaria alternata*, plant products, bio-control agents

INTRODUCTION

Onion (*Allium cepa* var., *aggregatum*) the queen of kitchen, considered as the poor man's staple spice, is flying out of the reach of even middle class families, grown for its bulb and also medicinal values. Like the crops, several diseases, among which leaf blight causes heavy losses in yield of onion. Leaf blight (*Alternaria alternata)* is a major problem in production of onion. Management of onion leaf blight disease by various strategies to be involved. The studies on the control of leaf blight caused by *Alternaria* sp. in onion and other crops have been carried out by various workers (Babu, 1994; Basin and Katircioglu, 1994; Sastrahidayal *et al*., 1995; Mohan, 1996; Karthikeyan *et al*., 2008) by chemicals. Studies were conducted on the control of the disease by using fungicides (Kannan and Subbaraja, 1999; Srivastava *et al*., 1999). Substantial use of chemical pesticides induces problems of health and environmental hazards in agricultural system. So, for human, plants and natural products of antimicrobial activity are best birational alternative today (Tiwari *et al*., 2007). Over the last two decades, intensive effort has been made to discover chemically useful antibacterial or antifungal drugs of plant origin (Valsaraj *et al*., 1997; Perumal Samy *et al*., 1999).

Reduction in the use of chemical pesticides in agriculture has been encouraged for a sustainable agriculture which necessitates the use of alternative strategies in combating the plant diseases. To overcome these problems use of plant products having more number of antimicrobial compound and biocontrol agents to be involved in disease management.

It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethano medicinal use of the plants (Ncube *et al*., 2008). Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Lis-Balchin and Deans, 1996; Maoz and Neeman, 1998; Hammer *et al*., 1999).

More number of plant species has been reported to possess natural substances that are toxic to many fungi causing plant diseases (Amadioha, 2000: Kagale *et al*., 2004). Bioagents of late have been known to induce systemic resistance against several plant diseases (Jetiyanon, 2007; Choudary *et al*., 2009). Bioagents like *Pseudomonas* and *Bacillus* strains consistently provided systemic protection against multiple diseases in various crops (Jetiyanon, 2007). Several strains of *Pseudomonas* and *Bacillus* elicit significant reduction in the incidence or severity of various diseases on diversity of hosts by elicitation of ISR which has been demonstrated (Choudary *et al*., 2009). With this background, in the present study, plant products and antagonistic microorganisms were exploited for the effective management of fruit rot incidence in glass house and field conditions.

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MATERIALS AND METHODS

Preparation of talc-based formulation: The talc-based formulations of the individual bacterial strains were prepared by the following method described by Vidhyasekaran *et al*. (1997). Briefly, a loopful of bacteria was inoculated into the King's B broth (King *et al*., 1954) and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (25+28°C). One kg of talc powder was taken in a sterilized metal tray and its pH was adjusted to neutral by adding $CaCO_3$ at the rate of 15 g kg^{-1}. Ten grams of Carboxy Methyl Cellulose (CMC) was added to 1 kg of talc and mixed well and the mixture was autoclaved for 30 min on each of two consecutive days. The 400 mL of 48 h grown bacterial suspension containing 96×10^8 cfu mL^{-1} was mixed with carrier cellulose mixture under aseptic conditions. For *Trichoderma,* 50 mL of molasses yeast (molasses 2%, yeast 0.3%) broth, autoclaved flasks was inoculated with 5 mm mycelial bits of *T. viride* and incubated at 28+2°C for 15 days (Singh and Majumdar, 2001). The spore concentration was adjusted to 1.56×10^8 cfu g^{-1}. After drying (approximately 35% moisture content) overnight under sterile conditions, it was packed in a polypropylene bag, sealed and stored at room temperature (Jayarajan *et al*., 1999). At the time of application, the population of fungi in the formulation was 3.6×10^8 cfu g^{-1} of talc powder.

Preparation of plant extracts: The fresh plant materials (*Acorus calamus* Rhizome, *Allium sativum* bulb, *Catharanthus roseus/Mentha arvensis* and *Prosopis juliflora* leaf) were separately washed in fresh water and finally in sterile water. These were separately ground in sterile water at the rate of 1 mL g^{-1} of the plant material in a pestle and mortar. The extract was expressed by squeezing the macerate with sterilized cotton wool. It was strained through two layers of muslin cloth and finally through sterilized Whatman No.1 filter paper. The extract was then passed through seitz filter to free from bacterial contamination. This formed the standard plant extract solution (100%). This was further diluted to the required concentration with sterilized medium/distilled water (Shekhawat and Prasad, 1971).

Field experiment: The field experiment was conducted at Randomized Block Design (RBD) with 12 treatments and 3 replications during kharif season at onion growing areas in Paavurchathiram at Tirunelveli district of Tamil Nadu, to assess the efficacy of plant extracts/oils and antagonistic microorganisms against onion leaf blight disease caused by *A. alternata*. The Agrifound red onion variety was used as test crop. The plants were raised with a standard spacing of 45×10 cm and the plot size was 4×3 m. The treatments were as follows:

- *Bacillus subtilis* at 0.2%
- *Pseudomonas fluorescens* at 0.2%
- *Trichoderma viride* at 0.2%
- Palmarosa oil at 0.05%
- Palmarosa oil at 0.1%
- Neem oil at 3.0%
- *Acorus calamus* at 10.0%
- *Allium sativum* at 10.0%
- *Catharanthus roseus* / *Mentha arvensis* at 10.0%
- *Prosopis juliflora* at 10.0%
- Mancozeb at 0.2%
- Control

Triton-E at 0.1% mixed with water used as a sticker/emulsifier for all the treatments. For control plots, triton-E mixed with water used as spray. The spraying was given on the initial appearance of the disease and another at 15 days later. The disease intensity was recorded at 15 days after the second spraying. The following score chart of 0-9 grade chart (TNAU, 1980) used for scoring the disease.

Grade	Leaf area blighted (%)
0	Healthy
1	Less than 1% leaf area infected
3	1-10% leaf area infected
5	10-25% leaf area infected
7	25-50% leaf area infected
9	More than 50% leaf area infected

The percent disease index was calculated by using Mckinney (1923) formula:

$$PDI = \frac{\text{Total sum of numerical ratings}}{\text{Total number of leaves observed}} \times \frac{100}{y}$$

where, y is the maximum category value in the score chart.

Statistical analysis: The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI) Biometrics unit, the Phillippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease indices were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($p<0.05$ and $p<0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS

Twelve treatments including biocontrol agents, plant oils, plant extracts and chemicals were used. Each treatment by spraying and all the treatments were found to be superior over control. The results revealed that two spraying of neem oil (3%) given on onion plants first at

Table 1: Efficacy of antagonistic microorganisms, plant oils and plant extracts against leaf blight disease of Onion (Foliar sprays)

Treatments	Disease mean Grade	PDI *	Disease reduction (%)	Yield (kg plot^{-1})	Yield (kg ha^{-1})	Increase in yield (%)	Cost benefit ratio
B. subtilis (0.2%)	3.07	34.07(35.70)^{b}	44.55	9.25	7708.33	72.89	1:5.12
P. fluorescens (0.2%)	3.60	40.00(39.22)^{b}	34.90	8.38	6983.33	56.63	1:4.65
T. viride (0.2%)	4.53	50.33(45.20)^{c}	18.08	6.59	5491.67	23.19	1:3.64
Palmarosa oil 0.05%	3.20	35.55(35.69)^{b}	42.14	9.20	7666.67	71.96	1:4.92
Palmarosa oil 0.1%	2.33	25.89(30.54)^{a}	57.86	9.42	7850.00	76.07	1:4.70
Neem oil 3.0%	2.00	22.22(28.1)^{a}	63.83	9.50	7916.67	77.77	1:4.38
Acorus calamus 10%	3.13	34.78(36.11)^{b}	43.39	9.34	7783.33	74.58	1:5.14
Allium sativum 10%	3.40	37.78(37.90)^{b}	38.51	8.88	7400.00	65.98	1:4.89
Mentha arvensis 10%	3.20	35.55(36.55)^{b}	42.14	8.90	7416.67	66.35	1:4.90
Prosopis juliflora 10%	3.47	38.55(38.33)^{b}	37.26	8.50	7083.33	58.88	1:4.68
Mancozeb 0.2%	1.86	20.66(27.66)^{a}	66.37	9.72	8100.00	81.68	1:5.12
Control	5.53	61.44(51.64)^{d}	-	5.35	4458.33	-	1:3.12
CD (p = 0.05%)	4.783			0.1600			

Data in parentheses are arc sine transformed values, *Means followed by a common letter are not significantly different at 5% level by LSD, Mean of three replications

the appearance of the disease and the second spray at 15 days later recorded lowest Percent Disease Index (PDI) 22.22 as against control (63.83) and this treatment recorded yield of 7916.67 kg ha^{-1} as against 4458.30 kg ha^{-1} in the control. This was followed by Palmarosa oil (0.1%) and *A. calamus* rhizome extract (10%) ranked next by recording 25.89 PDI and 34.78 PDI, respectively. These treatments were next only to the fungicide, Mancozeb (0.2%) which recorded the least disease intensity (PDI-20.66) and also highest yield 8100 kg ha^{-1}.

The yield was increased when two sprayings of neem oil 3.0% (77.77%) were given which was followed by palmarosa oil 0.1% (76.07%) and *A. calamus* rhizome extract 10% (74.58%). The yield was reduced in *T. viride* (23.19%) sprayed plots. However, two sprayings of Mancozeb (0.2%) were significantly found to be highly effective in increasing the yield (81.68%) (Table 1).

DISCUSSION

The present study was supported by (Sujatha Bai, 1992) who reported that neem oil 1.0% was used for the reduction of fruit rot disease incited by *A. tenuis*.

A similar result was supported by Karthikeyan *et al.* (2006). Neem oil reduced the leaf blight disease of onion incited by *A. palandui* in pot culture and field condition.

Neem (*Azadiracta indica* L.) is widely used and well known tree and seed extracts and oils are commonly used to control the insects and pathogens. A high content of Azadiractin, its active ingredient can be found both in the oil and in the extract (Mordue and Nisbet, 2002). Govindachari *et al.* (1998) also studied the antifungal activity of neem oil towards *Drechslera oryzae, Fusarium oxysporum* and *Alternaria tenuis*. Neem oil yields various acids, sulphur, etc. Meliantiol and azadiractin are obtained from seeds and decatylimbin also contains quecetin and sitosterol. The fungicidal spectrum of *Azadirachta indica* has been attributed to azadiractin which belongs to C25 terpenoides (Subramanian and Srinivasa Pai, 1953). In tomato crops, neem oil and extract have been used to control of white flies (*Bemisia tabaci*) (Kumar and Poehling, 2006), nematodes, fungi (Abbasi *et al*., 2005) and also *P. infestans* (Rani *et al*., 2006). The present investigation of various botanicals inhibiting the growth of *A. alternata* is in line with the earlier findings (Amaresh, 2000; Singh and Majumdar, 2001; Rao, 2006; Pramod Kumar, 2007).

Mohan *et al*. (2002) stated that palmarosa oil at 0.1% and 3.0% to control the onion leaf blight disease. Alice (1984) also reported that *A. calamus* rhizome extract (10%) significantly reduced the blight incited by *A. brassicae* in field. The highest disease intensity was recorded in case of *T. viride* sprayed plots (50.33) and therefore this was the least effective against the disease.

CONCLUSION

In summary, spraying of neem oil was the most effective plant products for control of onion leaf blight disease causing *Alternaria alternata*. However, the extract should be used on appropriate concentration that is non toxic to the host plant bulbs.

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REFERENCES

Abbasi, P.A., E. Riga, K.L. Conn and G. Lazarovits, 2005. Effect of neem cake soil amendment on reduction of damping-off severity and population densities of plant-parasitic nematodes and soilborne plant pathogens. Can. J. Plant Pathol., 27: 38-45.

Alice, D., 1984. Studies on antifungal properties of some plant extracts. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Amadioha, A.C., 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. Crop Prot., 19: 287-290.

Amaresh, Y.S., 2000. Epidemiology and management of *Alternaria* leaf blight and rust of sunflower (*Helianthus annus* L.). Ph.D. Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India.

Babu, S., 1994. Studies on leaf blight of tomato (*Lycopersicom esculantum* mill) caused by *Alternaria solani* (E-11 and Mart). M.Sc. (Ag.) Thesis, Tamilnadu Agricultural University, Coimbatore, India.

Basin, H. and Y.Z. Katircioglu, 1994. Studies of *in vitro* antagonistic effect of some *Bacillus subtilis* isolates against important plant pathogenic fungi Turkia II. Proceedings of the 2nd Turkish National Congress of Biological Control, January 25-28, 1994, Izmir, pp: 109-118.

Choudary, C., C. Kumar, F. Gnad, M.L. Nielsen and M. Rehman *et al*., 2009. Lysine acetylation targets protein complexes and co regulates major cellular functions. Science, 325: 834-840.

Gomez, K.A. and K. Gomez, 1984. Statistical Procedure for Agricultural Research. John-Wiley and Sons, Inc., New York.

Govindachari, T.R., G. Suresh, G. Gopalakrishnan, B. Banumathy and S. Masilamani, 1998. Identification of antifungal compounds from the seed oil of *Azadirachta indica*. Phytoparasitica, 26: 109-116.

Hammer, K.A., C.F. Carson and T.V. Riley, 1999. Antimicrobial activity of essential oils and other plant extracts. J. Applied Microbiol., 86: 985-990.

Jayarajan, R., G. Ramakrishnan, D. Dinakaran and R. Sridhar, 1999. Development of product of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot disease. In: Biotechnology in India, Dwivedi, B.K. (Ed.). Bioved Research Society, Allahabad, India, pp: 25-36.

Jetiyanon, K., 2007. Defensive related enzymes response in plants treated with a mixture of *Bacillus* strains (IN937a and IN937b) against different pathogens. Biol. Control, 42: 178-185.

Kagale, S., T. Marimuthu, R. Nandakumar and R. Samiyappan, 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiol. Mol. Plant Pathol., 65: 91-100.

Kannan, R. and K.T. Subbaraja, 1999. Comparative evaluation of selected plant extracts and fungicides on the incidence of leaf blight of onion caused by *Alternaria alternata* (Fr.) Keissler. Pestology, 23: 31-33.

Karthikeyan, M., R. Bhaskaran, K. Radhika, S. Mathizhagan, R. Sandosskumar, D. Alice and R. Velazhahan, 2006. Biological control of leaf blight of onion by use of oil formulations. Indian J. Plant Prot., 34: 89-93.

Karthikeyan, M., K. Radhika, R. Bhaskaran, S. Mathiyazhagan, R. Sandosskumar, R. Velazhahan and D. Alice, 2008. Biological control of onion leaf blight disease by bulb and foliar application of powder formulation of antagonist mixture. Arch. Phytopathol. Plant Protec., 41: 407-417.

King, E.O., M.K. Ward and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab Clin. Med., 44: 301-307.

Kumar, P. and H.M. Poehling, 2006. Persistence of soil and foliar azadirachtin treatmetns to control sweetpotato whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomatoes under controlled (laboratory) and field (netted green house) conditions in the humid tropics. J. Pest Sci., 79: 189-199.

Lis-Balchin, M. and S.G. Deans, 1996. Antimicrobial effects of hydrophilic extracts of *Pelargonium species* (Geraniacee). Lett. Applied Microbiol., 23: 205-207.

Maoz, M. and I. Neeman, 1998. Antimicrobial effects of aqueous plant extracts on the fungi *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. Lett. Applied Microbiol., 26: 61-63.

Mckinney, H.H., 1923. A new system of grading plant diseases. J. Agric. Res., 26: 195-218.

Mohan, K., 1996. Management of onion (*Allium cepa* L.) leaf blight disease incited by *Alternaria palandui* ayyangar with special reference to biological control. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Mohan, K., T. Seetharaman and E.G. Ebenezar, 2002. Management of leaf blight disease of onion caused by *Alternaria porri* by plant extracts, plant oils and biocontrol agents. NHRDF News Lett., 21: 11-14.

Mordue, A.J. and A.J. Nisbet, 2002. Azadiractin from the neem tree *Azadiracta indica*: Its action against insects. Ann. Braz. Soc. Entomol., 29: 615-632.

Ncube, N.S., A.J. Afolayan and A.I. Okoh, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr. J. Biotechnol., 7: 1797-1806.

Perumal Samy, R., S. Ignacimuthu and D. Patric Raja, 1999. Preliminary screening of ethnomedicinal plants from India. J. Ethnopharmacol., 66: 235-240.

Pramod Kumar, T., 2007. Biological management of Alternaria blight of onion. M.Sc. Thesis, University of Agricultural Science, Dharwad, India.

Rani, A., M.N. Bhat and B.P. Singh, 2006. Efficacy of neem formulations against late blight of potato in sub-tropical plains. Crop Res., 31: 179-180.

Rao, M.S.L., 2006. Management of Alternaria leaf spot in sunflower. Annual Report 2005-2006, Directorate of Oilseeds Research, India, pp: 54.

Sastrahidayal, I.R., S. Djauhari, H.S. Mudjo and H.M. Pusa Sawani, 1995. Antogonism testing of fungal antagonists against the casual agent of a disease an onion. Agricola, 16: 61-64.

Shekhawat, P.S. and R. Prasad, 1971. Antifungal properties of some plant extracts: Inhibition of spore germination. Indian Phytopathol., 24: 800-802.

Singh, J. and V.L. Majumdar, 2001. Efficacy of plant extracts against *Alternaria alternata*, the incitant of fruit rot of pomegranate (*Punica granatum* L.). Indian J. Mycol. Plant Pathol., 31: 346-349.

Srivastava, P.K., B.K. Tiwari and K.J. Srivastava, 1999. Studies on integrated disease management of onion. NHRDF News Lett., 19: 7-9.

Subramanian, C.V. and K.V. Srinivasa Pai, 1953. Relation of nitrogen to growth and sporulation of *Fusarium vasinfectum* ATK. Proc. Plant Sci., 37: 149-157.

Sujatha Bai, E., 1992. Studies on fruit rot of chillies (*Capsicum annum* L.) caused by *Alternaria tenuis* Nees. M.Sc. Thesis, Tamil Nadu Agricultural University, Madurai, India.

TNAU, 1980. Score chart for crop diseases. Tamil Nadu Agricultural University, Coimbatore, India, pp: 56.

Tiwari, R.K.S., A. Singh, K. Das and A. Sinha, 2007. Efficacy of extracts of medicinal plants against *Rhizoctonia solani*. Ann. Plant Prot. Sci., 15: 460-539.

Valsaraj, R., P. Pushpangadan, U.W. Smitt, A. Adsersen and U. Nyman, 1997. Antimicrobial screening of selected medicinal plants from India. J. Ethnopharmacol., 58: 75-83.

Vidhyasekaran, P., R. Rabindran, M. Muthamilan, K. Nayar, K. Rajappan, N. Subramanian and K. Vasumathi, 1997. Development of a powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol., 46: 291-297.