Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

SYNERGISTIC EFFECT OF PLANT EXTRACTS AND FUNGICIDE AGAINST PURPLE BLOTCH DISEASE OF ONION

Rabia Kalsoom[1], Sobia Chohan[2], Muhammad Saleem Haider[1], Muhammad Abid[2]
[1] *Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.*
[2] *Department of Plant Pathology, Bahauddin Zakariya University, Multan, Pakistan.*

ARTICLE INFO

Article history
Received: 13th May, 2019
Revised: 29th July, 2019
Accepted: 28th August, 2019

Keywords
Antifungal activity
Purple blotch of onion
Poisoned food technique
Alternaria porri

ABSTRACT

Onion is widely cultivated crop and attacked by different fungi. Purple blotch of onion caused by *Alternaria porri* is a continuous threat to Pakistan agriculture industry for the last few years. Generally, fungicides give satisfactory control but due to the environmental concerns, their use should be restricted. In integrated disease management program, cost effective and eco-friendly approach is used to cope with plant pathogens. Therefore, the present study was planned to evaluate the antifungal efficacy of different plants extracts such as *Pongamia pinnata, Polyalthia longifolia, Cassia fistula, and Citrus sinensis* against *A. porri* alone as well as synergistically with different concentrations of Mancozeb under *in vitro* conditions. Methanolic leaves extracts of these plants were mixed with different concentrations (25, 50, and 75%) of fungicide and screened via poisoned food technique to measure percent inhibition of fungal growth. The results indicated that plant extracts alone gave appreciable amount of fungal reduction but when used in combination with fungicide, performed best. Among them *P. longifolia* gave 73%inhibition, *C. sinensis* 49%, *P. pinnata* 70%and *Cassia fistula* showed46% inhibition respectively. In combination with fungicide, 25% of recommended dose, *P. longifolia* gave 82.93%, *P. pinnata* gave 77.07 %, *C. sinensis* 73.17% and *C. fistula* gave 62.92% inhibition as compared to control against *A. porri*. With the increase of fungicide in combination, more reduction in the fungal growth was observed. It is concluded that extracts can be used synergistically with fungicides. The combinations will protect the environment by reducing the amount of fungicide.

Corresponding Author: Sobia Chohan
Email: sobiachohan@bzu.edu.pk

INTRODUCTION

Onion belongs to genus *Allium* and is the essential part of our diet. It is the biannual or perennial plant and can be used both in raw and cooked forms. Beside nutritional value it is also known for pharmaceutical importance and home remedies. Onion is a great source of different vitamins such as vitamin A and C, minerals such as calcium, manganese and iron, dietary fiber and energy (Pareek et al., 2017; Priya et al., 2015). It is cultivated in at least 175 countries of the world for the last 5000 years. Onion is extensively grown on an area of 4,364,000 ha in whole world and its production is 74,250,809 tons.

Pakistan is at 7th position in onion production which is grown on an area of 135912 ha with the production 1739054 tons (FAO., 2015).

More than 60 diseases attack onion but the most destructive fungal disease is purple blotch of onion caused by *Alternaria porri*. The disease attacks every onion growing pocket of the world and 5 to 50% losses have been recorded due to this disease in all over the world (Maini et al., 1985). The disease first attacks the leaves and foliar parts of the plant and later on influences the maturity of the bulb. The disease produces the white necrotic spots on onion leaves. As the disease progresses, these white spots become large, zonate and develop reddish or purplish color at the margins of the lesions. It also influences the umbel stalk and girdles it which results in failure in seed production in case of severe infection. The disease also delays the maturity of the bulb and causes losses in yield of crop. It also makes the plant and bulb susceptible to other diseases.

Fungicides are very effective and give satisfactory control, but they have variety of problems like residual toxicity, environmental pollution and development of resistance in the pathogens (Anggriani et al., 2015). It is the need of the time to control the disease with less harmful effect of environment. Integrated disease management program has laid emphasis on the use of eco-friendly, cost effective and easily available compounds like plant extracts and bioagents to manage the disease (De Corato et al., 2014). Many plants are rich source of antimicrobial compounds which can be effective against pathogens. They can be used alone or in combination with others such as micronutrients, manures, bio-fertilizers and fungicides. Extracts of medicinal plants minimize the health hazards produced due to synthetic chemicals or fungicides (Chethana et al., 2015).Therefore, the present study was planned to save the environment by reducing the reliance on synthetic fungicides used to control the diseases. This could be done with the synergistic effect of natural components like plant extracts mixed with fungicides.

MATERIALS AND METHODS

Isolation of fungus: Disease samples of onion were collected from different fields of Multan. The leave portions showing characteristic symptoms of purple blotch were cut into small pieces (2-3cm), surface sterilized with 5% sodium hypochlorite. The pieces were placed under aseptic conditions on Petri plates having 10-15 ml potato dextrose agar medium for procurement of associated fungi. Isolated fungus was purified and stored in slants at 4^0C for further usage. Isolated fungus was identified as *A. porri* on morphological basis.

Fungicide: Mancozeb was purchased from pesticide Distribution Company in Multan. Different concentrations (25, 50, 75 and 100%) of fungicide were made by suspending it in distilled water for *in vitro* studies.

Collection and preparation of plant extracts: Plants belonging to four different families like Papilionacease, Rutaceae,Annonaceae and Fabaceae were selected for the study (Table 1). Fresh and healthy leaves of these plants were collected from nearby areas of Multan. The collected leaves were washed with tap water shade dried and then homogenized into fine powder. Plant extracts were prepared according to the method suggested by Abid et al. (2017) with slight modification. Powdered material (10 g) was extracted with 100 ml absolute methanol solvent for 48 hours in Soxhlet apparatus. The extracts were centrifuged at 6000 rpm for 10 min, filtered through Whatman No.1 filter paper and evaporated over a water bath at 40°C. After complete solvent evaporation, resultant crude extracts were stored in dark bottles at -20°C for further use. Stock solutions of extracts were prepared in Dimethyl Sulphoxide (DMSO).

Table 1. Antagonistic plants used in the study.

Local Name and English Names	Scientific Name	Family
Ulta Ashok or False Ashoka	*Polyalthia longifolia*	Annonaceae
Lemon	*Citrus limon*	Rutaceae
Sokh Chain or Indian Beech tree	*Pongamia pinnata*	Papilionaceae
Amaltas or Golden shower plant	*Cassia fistula*	Fabaceae

***In vitro* assessment of antifungal potential of medicinal plants:** The inhibitory effect of plant extracts alone and with different concentrations of fungicide was measured by following poisoned food technique (Nene and Thapilyal, 2002). For this, 1 ml of each plant extract was mixed in the potato dextrose agar (PDA) medium in Petri plates separately. The extracts were then mixed with different concentrations (25, 50 and 75 %) of

fungicide separately. The mixtures were poured and mixed in Petri plates containing PDA medium. After solidification, fungal plugs of 7mm size were placed in center of Petri plates. Plates having recommended dose (100%) of fungicide served as positive control while negative control had only distilled water. All the experiments were done under aseptic conditions and replicated thrice. Completely randomized design was applied. There were three replications for each treatment. The plates were further incubated at 28±2° C in an incubator with alternate light/darkness conditions. Percent inhibition was calculated using formula described by Vincent (1947):

$$I = \frac{(C - T)}{C} \times 100$$

Where 'I' is percentage growth inhibition, "C' is mean diameter (cm) of fungal colony in control and "T" is mean diameter (cm) of fungal colony with plant extract.

Statistical analysis: All the data were subjected to Analysis of Variance using SAS (SAS/IML software; Version 6; SAS, Institute) program (Steel et al., 1997). Means were separated by using Tukey's HSD test.

RESULTS AND DISCUSSION

Use of the fungicides to control diseases is the rapid and most effective way. Purple blotch of onion caused by *A. porri* could also be controlled by fungicides (Futane et al., 2018; Priya et al., 2015). In this study, Mancozeb under the trade name of Dominus 80% WP was used. It was observed that fungicide completely inhibited the fungal growth and gave 100% inhibition. Many previous studies have shown the efficacy of this fungicide against *A. porri*. Chethana et al. (2015) used many fungicides against purple blotch of onion and found mancozeb the most effective while Bavistin DF was found best by Rahman et al. (2015). Nainwal and Vishunavat (2016) also found that mancozeb was very efficient in controlling the disease. These studies strongly confirm the present findings.

Nevertheless, the use of the fungicide is not recommended due to their harmful aspects (Anggriani et al., 2015). It is the need of the time to find out the strategies to minimize the reliance on fungicide use. The less the quantity of chemical use, the less the environment gets damaged. With this concept many combinations had been formed and observed such as use of fungicides with micronutrients (Ali et al., 2016), use of fungicides with organic manure or fertilizers (Rashid et al., 2015), use of fungicides with plant extracts (Deepa et al., 2013) to control the fungal growth. This strategy was adopted in use of fungicide with plant extracts.

Many medicinal plants could also retard the growth of the pathogenic fungi. They have natural antimicrobial compounds as their self-defensive strategies. These compounds are formed in the process of evolution as nature has been changed day by day. They are natural compounds and are safe to use and leave no hazardous effect on environment, animals as well as human beings (Gurjar et al., 2012).

Four selected plants *P. longifolia*, *C. sinensis, P. pinnata* and *C. fistula* had already been reported for their antifungal activity against many fungi. These extracts do not inhibit mycelial growth completely just like fungicide. When methanolic extracts of leaves of four plants were used against *A. porri,* they gave noticeable results. *P. longifolia* gave 64% inhibition, *C. sinensis* 55%, *P. pinnata* 46%and *C. fistula* showed 43% inhibition against *A. porri* respectively (Table 2). Meena et al. (2013) reported the inhibition of radial growth of fungi by 74 and 73% by using *P. pinnata* and *P. longifolia* respectively. Gaikwad et al. (2014) observed that 100% concentration of *Lawsonia alba* was the most effective in controlling the growth of the *A. porri.* When these plant extracts were mixed with low doses of fungicides than the recommended ones, they acted synergistically. Synergism states as the action of two dissimilar agents working together and generate superior results than their independent effect.

Our work successfully demonstrated this between fungicide and plants extracts. Plant extracts efficiently inhibited the growth of the pathogen by using the much lower quantity of the fungicide than the recommended dose. In combination with fungicide, 25% of recommended dose, *P. longifolia*gave70%, *P. pinnata* gave 64 %, *C. sinensis* 63% and *C. fistula* gave 55% inhibition as compared to control against *A. porri*. When the quantity of fungicide was increased to 50% and 75% in the mixture more inhibition was observed as described in Table 2.

At the lower dose of 25 %, the plant extracts gave 100% inhibition in case of *C. fistula* while *C. sinensis* gave 97%, *P. longifolia* gave 93% and *P. pinnata* gave 88% inhibition. Fielding et al. (2015) also tested eight plant extracts with kresoxim-methyl for the management of *Botrytis cinerea* in apples. They observed the synergistic and effective control with plant extracts and fungicides. Deepa et al. (2013) also strongly recommended this type of synergy between plant extracts and fungicide to reduce the doses of fungicides in chemical treatment of seeds.

Table 2: Effects of plant extracts and fungicide on the growth of *Alternaria porri.*

Plant extracts	Mycelial growth (mm)	% inhibition
Negative control (without any treatment)	7.9	0.00±0.66
100% fungicide or positive control	0.00	100.00±00
Polyalthia longifolia	2.63	73.17± 0.19
25% of fungicide + *P. longifolia*	2.01	82.92±0.14
50% of fungicide + *P. longifolia*	1.76	90.56±0.11
75% of fungicide + *P. longifolia*	0.98	93.07±0.04
Pongamia pinnata	3.45	70.4 ±0.26
25% of fungicide + *P. pinnata*	2.35	77.07±0.16
50% of fungicide + *P. pinnata*	1.88	85.87±0.13
75% of fungicide + *P. pinnata*	1.53	88.78±0.08
Citrus sinensis	4.75	49.51±0.377
25% of fungicide + *citrus*	3.20	73.17±0.27
50% of fungicide + *citrus*	2.90	78.04±0.25
75% of fungicide + *citrus*	1.12	97.56±0.04
Cassia fistula	4.35	46.58±0.34
25% of fungicide + *C. fistula*	3.61	62.92±0.32
50% of fungicide + *C. fistula*	1.45	90.73±0.10
75% of fungicide + *C. fistula*	0.00	100.00±00

Data are means of three replicates. **±** Standard Deviation.

At the lower dose of 25 %, the plant extracts gave 100% inhibition in case of *C. fistula* while *C. sinensis* gave 97%, *P. longifolia* gave 93% and *P. pinnata* gave 88% inhibition. Fielding et al. (2015) also tested eight plant extracts with kresoxim-methyl for the management of *Botrytis cinerea* in apples. They observed the synergistic and effective control with plant extracts and fungicides. Deepa et al. (2013) also strongly recommended this type of synergy between plant extracts and fungicide to reduce the doses of fungicides in chemical treatment of seeds.

Conclusion: It is concluded that mixture of low doses of fungicide and plant extracts worked synergistically and made the efficient formulation to keep the inoculum under the thresh hold level with less harm to the environment.

Author Contributions: RK, SC and MSH planned and designed the studies, RK executed the experiments and collected data, SC and MSH supervised the research work, MA analyzed the data, RK wrote the manuscript and all the authors edited it.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgements: The work is a part of ongoing Ph.D. degree funded by Higher Education Commission of Pakistan.

REFERENCES

Abid, M., Chohan, S., Mehmood, M.A., Naz, S., Naqvi, S.A.H., 2017. Antifungal potential of indigenous medicinal plants against Myrothecium leaf spot of bitter gourd (*Momordica charantia L.*). Brazilian Archives of Biology and Technology 60, v.60: e17160395 Jan/Dec 17162017.

Ali, H., Nisha, H.A.C., Hossain, M.B., Islam, M.R., 2016. Evaluation of combined effect of micronutrients (ZnSO4+ Borax) and fungicides to control the purple blotch complex of onion (*Allium cepa*). American Journal of Plant Sciences 7, 715.

Anggriani, N., Putri, L.N., Supriatna, A.K., 2015. Stability analysis and optimal control of plant fungal epidemic: An explicit model with curative factor, AIP Conference Proceedings. AIP, pp. 40-47.

Chethana, B.S., Ganeshan, G., Archana, S.R., Bellishree, K., 2015. Integrated disease management of purple blotch disease of onion. The Bioscan 10, 1729-1733.

De Corato, U., Viola, E., Arcieri, G., Valerio, V., Cancellara, F.A., Zimbardi, F., 2014. Antifungal activity of liquid waste obtained from the detoxification of steam-exploded plant biomass against plant pathogenic fungi. Crop Protection 55, 109-118.

Deepa, N., Nayaka, S.C., Girish, K., Raghavendra, M.P., 2013. Synergistic effect of *Prosopis juliflora* extract and chemical fungicides against seed borne toxigenic fungi. International Journal of Advanced Life Sciences 6, 312-317.

FAO., 2015. Production Year Book, Food & Agricultural Organization, Rome.

Fielding, B.C., Knowles, C., Vries, F.A., Klaasen, J.A., 2015. Testing of eight medicinal plant extracts in combination with Kresoxim-Methyl for integrated control of *Botrytis cinerea* in apples. Agriculture 5, 400-411.

Futane, A.S., Dandnaik, B.P., Salunkhe, S.S., Jadhav, P.P., Magar, S.J., 2018. Management of storage diseases of onion by using different fungicides and antibiotics. International Journal of Current Microbiology and Applied Sciences 7, 1149-1158.

Gaikwad, K.N., Jadhav, Shobha, U., Kakulte, V.R., 2014. Management of fungal diseases of onion (*Allium cepa L.*) by using plant extract. International Journal of Life Sciences and Pharma Research 4, 28-30.

Gurjar, M.S., Ali, S., Akhtar, M., Singh, K.S., 2012. Efficacy of plant extracts in plant disease management. Agricultural Sciences 3, 425.

Maini, P.K., Murray, J.D., Oster, G.F., 1985. A mechanical model for biological pattern formation: a nonlinear bifurcation analysis, in: Sleeman, B.D., Jarvis, R.J. (Eds.), Ordinary and Partial Differential Equations. Springer, pp. 252-269.

Meena, A.K., Khan, S., Rao, M.M., Reddy, R.K., Padhi, M.M., 2013. Antifungal metabolites from medicinal plants used in Ayurvedic System of medicine in India, in: Razzaghi-Abyaneh M., Rai, M. (Eds.), Antifungal Metabolites from Plants. Springer, Berlin, Heidelberg.

Nainwal, D., Vishunavat, K., 2016. Management of purple blotch and Stemphylium blight of onion in Tarai and Bhabar regions of Uttarakhand, India. Journal of Applied and Natural Science 8, 150-153.

Nene, Y., Thapilyal, L., 2002. Fungicides in plant disease control. . Oxford and IBH Publishing Company, New Delhi, p. 507.

Pareek, S., Sagar, N.A., Sharma, S., Kumar, V., 2017. Onion (*Allium cepa* L.).

Priya, R.U., Sataraddi, A., Darshan, S., 2015. Efficacy of non-systemic and systemic fungicides against purple blotch of onion (*Allium cepa L.*) caused by *Alternaria porri* (Ellis) Cif. International Journal of Recent Scientific Research 6, 6519-6521.

Rahman, S.M., Maniruzzaman, S.M., Nusrat, S., Khair, A., 2015. In vitro evaluation of botanical extract, bioagents and fungicides against purple blotch diseases of bunch onion in Bangladesh. Advances in Zoology and Botany 3, 179-183.

Rashid, M.H., Haque, M.M., Bakr, M.A., Islam, M.R., 2015. Effect of Chemicals and Environment Friendly Components on Growth Parameters and Yield Contributing Character of Onion (*Allium cepa*). Journal of Agriculture and Food Technology 5, 8-14.

Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and Procedures of Statistics. A Biometrical approach, 3rd ed. McGraw Hill Book Co., New York, USA.

Vincent, J.M., 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 159, 850.