Efficacy of Bidens pilosa and Euphorbia hirta Extracts in Control of Bacterial Leaf Spot Disease of Solanum scabrum

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***Abstract*: Bacterial leaf spot disease incited by *Xanthomonas campestris* pv. *vescatoria* affects solanum plants worldwide and caused up to 40-70% yield loss of *Solanum scabrum*. Efficacy of *Bidens pilosa* and *Euphorbia hirta* leaf and root extracts and one synthetic chemical Ridomil® was evaluated for control of bacterial leaf spot in *Solanum scabrum*. The experiment was laid out in randomized complete block design with three replicates. Seedlings were inoculated with *Xanthomonas campestris* pv. *vesicatoria* isolated from diseased *S. scabrum* and then treated separately with test concentrations of 25%, 50%, 100% and 50 mg/ml 100 mg/ml and 200 mg/ml for water and ethanol extract respectively. The extracts reduced disease severity with the highest concentrations (100% and 200 mg/ml significantly lowering disease severity compared to other extracts and Ridomil®. Plants treated with high concentrations of extracts had high growth vigor when stem diameter, plant height and leaf weight were evaluated. Reduction in disease severity and promotion of plant growth could be due to presence of secondary metabolites with antimicrobial activity and growth promoting hormones in the extracts. From this study *B. pilosa* and *E. hirta* extracts can be used as an alternative to synthetic chemical to control bacterial leaf spot disease of solanum. Future studied should focus on isolating active compounds to be used in formulating pesticides.**

***Key words:* Bacterial leaf spot, Xanthomonas campestris pv. vesicatoria, Biden pilosa, Euphorbia hirta, efficacy.**

I. INTRODUCTION

Bacterial leaf spot is a disease that greatly affects plants in solanaceae family including tomato

(*Solanum lycopersicum*), capsicum and chilli (*Capsicum annuum*) and nightshades (*Solanum scabrum*) (Roach *et al*., 2017; Emitaro *et al*., 2017; Potnis *et al.*, 2015). In *Solanum scabrum*, the disease is caused by *Xanthomonas campestris* pv.*vesicatoria* bacterium that is gram negative, aerobic rod-shaped and non spore forming (Emitaro *et al*., 2018; AL-Saleh, 2011).The inoculums for the disease originates from diseased plant debris, infected seeds and volunteer plant crops where it is disseminated by rain drops and sprinkler water driven by wind to susceptible plants (Roach *et al*., 2018; Araújo *et al*., 2012). Bacterial leaf spot disease has been reported to cause up to 40-70% crop loss of nightshades and other related plants in East Africa (Emitaro *et al*., 2017; Mbega *et al.*, 2012).

Farmers control bacterial leaf spot disease using cultural methods, antagonistic bacteria and by applying copper based synthetic bactericides and fungicides (Perez *et al*., 2019; Kebede *et al.*, 2013). However, application of synthetic chemicals is being discouraged because of toxic residues that persist in the environment thus causing adverse health and environmental effects. Moreover, these chemicals are not affordable or easily accessible to vegetable growers in developing countries (Hossain *et al*., 2018; Le *et al*., 2020). Because of the deleterious effect of synthetic chemicals, there is a need to seek for alternative methods of controlling bacterial leaf spot with minimal adverse effect on health and environment.

Many naturally occurring plants both medicinal and none medicinal have useful properties for crop protection but their potential in controlling plant pathogens have not been explored (Hossain *et al*., 2018; Syed-Ab-Rahman *et al*., 2020; Verma and Agrawal 2017). Natural products especially secondary metabolites from plants have been reported to have antimicrobial properties, affordable, easily available, harmless to humans and animals and cause little disturbance to ecological balance (Verma and Agrawal 2017; Didwania *et al*., 2013). Plants like *Bidens pilosa* and *Euphorbia hirta* have shown great toxicity against plant pathogens including insect pests, fungi and bacteria. *Bidens pilosa* extracts have been used to control aphids, cutworm and termites in tea (Mamun and Ahmed, 2011) while *E. hirta* extracts have shown antimicrobial activity against *Aspergilus flavus* (Gayathri and Ramesh, 2013). Even though extracts from these two plants have been found to be active against some plant pathogens, their efficacy against *Xanthomonas campestris pv.vesicatoria* and their use in management of bacterial leaf spot in *Solanum scabrum* is yet to be documented. This study therefore was to determine the potential of leaf and root extracts of *Biden pilosa* and *Euphrbia hirta* in controlling bacterial leaf spot affecting *Solanum scabrum* in order to improve its yield.

II. MATERIALS AND METHODS

Plant Material Collection and Test Concentration Preparation

Leaves and roots of *Bidens pilosa* and *Euphorbia hirta* were collected separately around Bondo campus, Jaramogi Oginga Odinga University of Science and Technology and identified by plant taxonomist in botany laboratory. They were thoroughly washed with tap water, dried under shade for 14 days and then powdered using an electric motor. Fine powder collected was used for extraction in water and ethanol solvents. 10 grams of powdered leaf and root materials were separately kept in 500 ml conical flask and 100 mL of distilled water and ethanol added respectively. The conical flasks were covered with aluminum foil, mixed thoroughly and left to stand overnight for complete elucidation of active materials. The extract was filtered using muslin cloth followed by Whatman no 1 filter paper. Ethanol was evaporated using rotary vacuum evaporator with the water bath at the temperature of 45 ˚C. The filtrates were used to make different concentrations of 25%, 50% and 100% water extract and 50 mg/mL, 100 mg/mL and 200 mg/mL for ethanol extract. Ridomil® was used as standard as recommended by the manufacturer while plain water was used an as control (Shandukani *et al*., 2018).

Isolation of the Pathogen from Infected S. scabrum leaves

Six samples of infected leaves of *S. scabrum* showing water-soaked and necrotic areas were collected from farmer's field in Bondo and taken to microbiology laboratory for isolation according to Opara and Obani procedure as reported by Emitaro *et al.* (2018). The leaf samples were washed separately in sterile water, surface-sterilized in 70% ethanol and rinsed in several changes of sterile water. Small pieces of the infected portions were cut from the margin of the lesion using sterile scalpel then teased apart with sterile dissecting needle in 1 mL sterile water and left to stand for 30 minutes. The suspension was then streaked onto the surface of modified Tween B media and then incubated at 30 ˚C for 48 hours. The isolates were morphologically and biochemically identified according to Gracelin *et al*., (2012) and AL-Saleh (2011) methods and purified on Yeast dextrose calcium carbonate (YDC) media.

Soil and Nursery Preparation

Top moist soil was collected from the nursery site and mixed with farm yard manure to improve its organic matter content. Soil mixture was moistened, put into a cut drum, covered and heated to a temperature of 80 ˚C for 1hr 30 minutes. It was allowed to cool before transferring into 4 pots of 3 liters and about 20 cm diameter. Soil in pots was moistened, seeded with seeds obtained from the agro-veterinary and watered twice daily. Pots were covered with mulching material to conserve moisture. Four days after germination, mulch material was removed to prevent damping off and death of the seedlings (Opara and Obani 2010). Seedlings were left in the pots for seven days after germination then transplanted into green house plots.

Greenhouse Preparation, Transplanting and Experimental Design

Greensil method as reported by Opara and Obani (2010) was used. The field was ploughed into fine tilth, Plots of 0.5 m x 0.5 m prepared and each plot was transplanted with 10 seedlings. A total of 42 plots were prepared 18 for *B. pilosa* leaf and root extract, 18 for *E. hirta* leaf and root extract, 3 for sterile water as control and 3 for Ridomil® as standard. Leaf and root extracts of *B. pilosa* and *E. hirta* had three treatments each, 25%, 50% and 100% for water and 50 mg/mL, 100 mg/mL and 200mg/mL for ethanol, which were replicated three times. The plots were arranged in randomized complete block design (RCBD), watered twice daily and weeding done by hand picking weeds regularly.

Pathogenecity Test

Four weeks old *Solanum scabrum* seedlings leaves were infiltrated with about 1mL of 10^{-4} suspension of the bacterial inoculum on the underside using hypodermic syringe without a needle. One milliliters of sterile water was also infiltrated in another seedling as negative control and left for 48 hours to test for the virulence of the bacterial organism according to Opara and Obani (2010). The inoculated plants were covered in plastic bags to maintain humidity at its maximum (Gracelin *et al*., 2012).

Seedling Inoculation and Treatment Application

Field inoculation was done on 2 weeks old seedlings in the plots arranged in randomized complete block design replicated three times in a green house. Nutrient broth was inoculated with pure colonies from YDC and incubated for 48 hours. Bacterial suspension was prepared by serial diluting the broth to 10^{-4} in sterile water in ratio of 1:9. The seedlings were inoculated by spraying 500 mL of bacterial suspension per plot in the evening using a hand sprayer. Incubation period of 48 hours was given to allow the bacteria to infect the seedlings before spraying them with different concentration of the extracts. Each plot was sprayed with a different concentration without mixing the concentrations until the plots were soaked (Opara and Obani 2010). Ridomil® and sterile water were also sprayed to infected seedling using hand sprayer as control and standard respectively.

Disease Severity Assessment.

Severity scores were based on the first four leaves from the youngest open foliage using the scale 0-6 as outlined by Opara and Obani (2010).

0 = Leaves without spots
1 = 1-3 spots on leaves
2 = 1/5 of the leaves covered with spots
3 = 1/3 of the leaves covered with spots
4 = ½ of the leaves covered with spots
5 = 2/3 of the leaves covered with spots

6 = the entire leaf area affected

Evaluation of Growth Parameters and Yield

Growth parameters data was collected from two weeks after transplanting (Ondieki *et al.,* 2011). Five plants per experimental plot were randomly selected and tagged for data collection.

Plant height (cm) was measured using a meter rule while stem diameter (mm) was determined using micrometer screw gauge. Yield was determined by measuring the fresh leaf weight in grams using field electronic balance. Data was analyzed using SAS software (SAS Institute, version 9.1). The data was subjected to analysis of variance (ANOVA) and where significant, means were separated using Fisher's Least Significant Difference at P≤0.05.

III. RESULTS

Water and ethanol extracts of both *B. pilosa* and *E. hirta* significantly reduced disease severity. In water extracts, *E. hirta* significantly (P≤0.05) reduced disease severity than *B. pilosa* while in ethanol extracts there was no significant (P≥0.05) difference in disease severity reduction between the two plant extracts (Table 1). Leaf and root extracts reduced disease severity with no significant (P≥0.05) difference for both water and ethanol extract except for *B. pilosa* water extract where there was significant (P≤0.05) difference with root extracts reducing disease severity than leaf extract (Table 2). Different concentrations of leaf and root water extracts of *E. hirta* and roots of *B. pilosa* had no significant (P≥0.05) difference in disease severity reduction. Different concentrations of water leaf extract of *B. pilosa* significantly reduced disease severity with higher concentration (100%) giving the lowest severity index compared to other concentrations (Table3). Leaf and root ethanol extracts of *E. hirta* and root extracts of *B. pilosa* significantly reduced disease severity with higher concentration (200 mg/mL) producing lowest severity index than other concentrations. Different concentration of *B. pilosa* ethanol leaf extracts had no significant different in disease severity reduction (Table3). Similarly there was no significant difference in disease severity reduction between different concentrations and synthetic chemical Ridomil (Table3).

Table 1: Effect of B. pilosa and E. hirta water and ethanol extract on disease severity.

Plant species	Water extract	Ethanol extract
B. pilosa	2.47^{a}	2.18^{a}
E. hirta	2.20^{b}	2.05^{a}
P value	0.007	0.125
LSD	0.188	0.16

Means with different superscript letters along the column are significantly different (P<0.05).

Table 2: Effect of B. pilosa and E. hirta leaves and roots water and ethanol extract on disease severity

Plant parts	B. pilosa		E. hirta	
	Water extract	Ethanol extract	Water extract	Ethanol extract
Leaf	2.62^{a}	2.25^{a}	2.27^{a}	1.98^{a}
Root	2.32^{b}	2.10^{a}	2.15^{a}	2.13^{a}
P value	0.015	0.204	0.317	0.168
LSD	0.241	0.233	0.23	0.238

Means with different superscript letters along the column are significantly different (P<0.05).

Table 3: Effect of different concentrations of B. pilosa and E. hirta extract on disease severity

Water extract [%]						Ethanol extract [mg/mL]				
Conc.	B. pilosa		E. hirta			Conc.	B. pilosa		E. hirta	
	Leaf	Root	Leaf	Root			Leaf	Root	Leaf	Root
0%	4.22^{a}	4.22^{a}	4.22a	4.22^{a}		0mg/ml	4.42^{a}	4.42^{a}	4.22^{a}	4.22^{a}
5%	2.75^{b}	2.00^{b}	2.00^{b}	1.58^{b}		25mg/ml	2.00^{b}	1.75^{bc}	1.83^{b}	1.92^{b}
50%	2.33^{bc}	1.59^{b}	1.58^{b}	1.58^{b}		100mg/ml	1.50^{b}	1.42^{bc}	1.25^{b}	1.33^{bc}
100%	1.75^{c}	1.75^{b}	1.50^{b}	1.33^{b}		200mg/ml	1.50^{b}	1.17^{c}	0.50^{c}	1.17^{c}
Ridomil	1.83^{c}	1.83^{b}	1.83^{b}	1.83^{b}		Ridomil	1.83^{b}	1.83^{b}	1.83^{b}	1.83^{b}
P value	<.0001	<.0001	<.0001	<.0001		P value	<.0001	<.0001	<.0001	<.0001
LSD	0.622	0.641	0.667	0.574		LSD	0.649	0.596	0.639	0.642

Means with different superscript letters along the column are significantly different (P<0.05).

There was significant effect of the treatments on growth parameters determined. Water extract of *B. pilosa* and *E. hirta* increased stem diameter and plant height with no significant difference while leaf weight was increased with significant difference. Ethanol extracts of *B. pilosa* and *E. hirta* significantly increased stem diameter, plant height, and leaf weight (Table 4). There was no significant difference in the diameter of the stems and leaf weight of the plants treated with water extracts of leaves and roots of both *B. pilosa* and *E. hirta* while root water extracts caused an increase in plant height more than leaf extract. Plants treated with leaf and root ethanol extract of *B. pilosa* had no significant difference in stem diameter whereas there was significant difference in plant height and leaf weight of the same plants (Table 5). At different concentrations, the highest concentrations 100% and 200 mg/ml for water and ethanol extract respectively caused significant increase in plant stem diameter, plant height and leaf weight compared to other concentrations and synthetic chemical Ridomil (Table 6).

Table 4. Effect of B. pilosa and E. hirta extract on stem diameter, plant height and leaf weight.

		Water extract [%]			Ethanol extract		[mg/mL]
Plant species		SD (cm)	PH (m)	LW(g)	SD (cm)	PH (m)	LW (g)
B. pilosa		6.58^{a}	20.91^{a}	1.68^{b}	6.64^{b}	20.72^{b}	1.73^{b}
E. hirta		6.6^{a}	21.05^{a}	1.72^{a}	6.70^{a}	20.97^{a}	1.80^{a}
P value		0.096	0.104	0.115	0.025	0.002	0.003
LSD		0.062	0.176	0.048	0.061	0.163	0.047

Means with different superscript letters along the column are significantly different (P<0.05).

Key: SD- stem diameter, PH- plant height, LW- leaf weight

Table 5. Effect of *B. pilosa* and E. *hirta* leaf and root extracts on stem diameter, plant height and leaf weight

	Water extract (%)						Ethanol extract (mg/mL)					
	B. pilosa			*E. hirta*			*B. pilosa*			*E. hirta*		
Plant parts	SD [cm]	PH [m]	LW (g)	SD [cm]	PH [m]	LW [g]	SD [cm]	PH [m]	LW [g]	SD [cm]	PH [m]	LW [g]
Leaf	6.57a	20.64^{b}	1.65^{a}	6.62^{a}	20.75^{b}	1.69^{a}	6.22^{a}	20.48^{b}	1.70^{a}	6.69^{a}	20.60^{b}	1.76^{b}
Root	6.58a	21.17^{a}	1.27^{a}	6.64^{a}	20.35^{a}	1.74^{a}	6.65^{a}	20.94^{a}	1.76^{a}	6.72^{a}	21.34^{a}	1.84^{a}
P value	0.738	<.0001	0.119	0.662	<.0001	0.192	0.489	<.0001	0.139	0.532	<.0001	0.02
LSD	0.091	0.252	0.068	0.086	0.267	0.071	0.088	0.224	0.07	0.086	0.253	0.067

Means with different superscript letters along the column are significantly different (P<0.05)

Key: SD- stem diameter, PH- plant height, LW- leaf weight

Table 6: Effect of different concentrations of B. pilosa and E. hirta water and ethanol extracts stem diameter, plant height and leaf weight

	Water extract		[%]					Ethanol exract					
	B. pilosa			*E. hirta*				*B. pilosa*			*E. hirta*		
Conc. [%]	SD [cm]	PH [m]	LW [g]	SD [cm]	PH [m]	LW [g]	Conc [mg/ml]	SD [cm]	PH [m]	LW [g]	SD [cm]	PH [m]	LW [g]
0	5.95^{d}	17.47^{e}	1.42^{d}	5.95^{d}	17.50^{e}	1.36^{d}	0	6.13^{d}	15.76^{e}	1.141^{e}	6.13^{d}	15.76^{e}	1.40^{d}
25	6.36^{c}	21.50^{c}	1.52^{d}	6.51^{c}	21.71^{c}	1.57^{c}	25	6.37^{c}	21.72^{c}	1.58^{d}	6.60^{c}	22.08^{c}	1.68^{c}
50	6.71^{b}	22.09^{b}	1.81^{b}	6.84^{b}	22.30^{b}	1.84^{b}	100	6.75^{b}	22.36^{b}	1.86^{b}	6.89^{b}	22.80^{b}	2.01^{b}
100	7.05^{a}	22.87^{a}	1.95^{a}	7.02^{a}	23.14^{a}	2.10^{a}	200	7.11^{a}	23.14^{a}	2.06^{a}	7.09^{a}	23.62^{a}	2.19^{a}
Ridomil	6.81^{b}	20.60^{d}	1.70^{c}	6.81^{b}	20.60^{d}	1.72^{b}	Ridomil	6.81^{b}	20.60^{d}	1.74^{c}	6.81^{b}	20.60^{d}	1.74^{c}
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
LSD	0.143	0.399	0.109	0.136	0.421	0.112	LSD	0.14	0.355	0.111	0.135	0.4	0.106

Means with different superscript letters along the column are significantly different (P<0.05)

Key: SD- stem diameter, PH- plant height, LW- leaf weight

IV. DISCUSSION

Plants have become the subject of research in attempt to identify alternative novel active compounds to control plant pathogens and manage plant diseases (Syed-Ab-Rahman *et al*., 2020; Verma and Agrawal 2017). Growth of a plant is a function of optimum metabolic activities with less interference from abiotic and biotic stress factors. Management of diseases in food crops ensures high yields while guaranteeing food security. Many farmers around the world have impressed the use of botanicals to control and manage plant disease as they are ecofriendly with least side effects and easily biodegradable (Choudhury *et al*., 2018; Salhi *et al*., 17). Extracts from *B. pilosa* and *E. hirta* reduced disease severity probably because they contain secondary metabolites that inhibited the multiplication of the pathogen within the plant. Inhibition of multiplication could be by interference with cell wall or protein synthesis thus arresting their growth. The results are in agreement with the report of Arefin *et al.* (2019) that plant extracts reduced severity of Alternaria blight disease of radish. Plant extracts contain secondary metabolites such as tannins, flavanoids, saponins and alkaloids that have antimicrobial properties (Emitaro *et al*., 2020; Nirosha *et al*., 2019; Izah *et al*., 2018). Higher concentrations of the extracts (100% and 200 mg/mL) reduced disease severity more than other concentration probably because the active ingredients worked synergistically to suppress the proliferation of the pathogen. The results concurs with those reported by Abo-Elyousr *et al*. (2020) that higher concentrations of the plant extracts reduced disease severity in tomato plants.

Plants sprayed with extracts had increased stem diameter, plant height and leaf weight probably because the extract inhibited the development of bacterial leaf spot disease that causes defoliation hence increasing photosynthetic surface. With adequate photosynthesis, there were adequate food reserves for the growth and development of the plant hence increased yield. Similar results were reported by Abo-Elyousr *et al*. (2020) and Din *et al*. (2016) that extracts increased growth characters and yield of tomato plants. Improved growth could also be attributed to the presence of growth promoting hormones in the extracts. Extracts from *Moringa oleifra* have been reported to contain growth hormones that stimulate growth and increase resistance of young plants against plant pathogens (Abdalla *et al*., 2013). Therefore this study revealed the effectiveness of using *B. pilosa* and *E. hirta* extract to control bacterial leaf spot of *Solanum scabrum*. Use of *B. pilosa* and *E. hirta* extracts could solve the problem of overdependence on synthetic chemicals to control bacterial leaf sport in order to improve yield of *Solanum scabrum* and other relates vegetables for food security.

V. CONCLUSION

This study aimed at determining the efficacy of extracts from *Biden pilosa* and *Euphorbia hirta* against bacterial leaf spot disease of tomatoes caused by *Xanthomonas campestris* pv. *vescatoria*. The extracts effectively reduced disease severity due to secondary metabolites they contained. The ability of extracts from *B. pilosa* and *E. hirta* to reduce disease severity could form a basis of developing botanical pesticides to avoid the adverse effects of synthetic chemicals. The results in this study supports the use of plant extracts in management of plant diseases as they are readily available, biodegradable, cheap and ecofriendly. Furthermore, the extracts contain growth promoting hormones that accelerates growth of the plant they are applied to. Future studies should focus on identifying the active ingredients in the extracts of *B. pilosa* and *E. hirta* for development of chemicals to optimize their use by smallholder farmers in disease control and growth promotion to reduce dependence on synthetic pesticides.

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REFERENCES

[1] Abdalla, M.M. (2013). The Potential of Moringa Oleifera Extract as a Biostimulant in Enhancing the Growth, Biochemical And Hormonal Contents In Rocket (Eruca vesicaria Subsp. sativa) Plants. International Journal of Plant Physiology and Biochemistry, (3), 42-49.

[2] Abo-Elyousr, K. A. M., Najeeb, M. A., Ahmed, W.M. A., Sergio R. R. and Khamis, Y. (2020). Plant Extract Treatments Induce Resistance to Bacterial Spot by Tomato Plants for a Sustainable System. Horticulturae, 6(36), 1-12.

[3] AL-Saleh, M. A. (2011). Pathogenic Variability among Five Bacterial Isolates of Xanthomonas campestris pv. vesicatoria, Causing Spot Disease on Tomato and their Response to Salicylic Acid. Journal of the Saudi Society of Agricultural Sciences, 10(1), 47-51.

[4] Arefin, M.N., Bhuiyan, M. K. A. and Rubaye, M.T. (2019). Integrated use of Fungicide, Plant Extract and Bio-Agent for Management of Alternaria Blight Disease of Radish (Raphanus Sativus L.) and Quality Seed Production," Research in: Agricultural & Veterinary Sciences, 3(1), 10-21.

[5] Araújo, E.R., Costa, J.R., Ferreira, M.A.S.V. and Quezado-Duval, A.M. (2012). Simultaneous Detection and Identification of the Xanthomonas Species Complex Associated with Tomato Bacterial Spot Using Species-Specific Primers and Multiplex PCR. Journal of Applied Microbiology, 113(6), 79-90.

[6] Choudhury, D., Prerna, D., Seweta, S., Soumen, S. and Susamoy, K. (2018). Role of Botanical Plant Extracts to Control Plant Pathogens-A review. Indian Journal of Agricultural Research, 52(4), 341-346.

[7] Didwania, N., Deepti, S. and Trivedi, P. C. (2013). Antibacterial Activity of a Few Medicinal Plants against Xanthomonas Campestris Pv . Campestris. International Journal of Research in Pharmaceutical Sicences, 4, 177–182.

[8] Din, N., Ahmad, M., Siddique, M., Ali, A., Naz, I., Ullah, N. and Ahmad, F. (2016). Phytobiocidal Management of Bacterial Wilt of Tomato Caused by Ralstonia solanacearum (Smith). Yabuuchi, Spanish Journal of Agricultural Research, 14(3), 1-13. http://dx.doi.org/10.5424/sjar/2016143-9012.

[9] Emitaro, W.O., Musyimi, D.M., Opand, G.T. and Odhiambo, G. (2020). Phytochemical and Antimicrobial Properties of Leaf Extracts of Calliandra calothyrsus, Leucaena diversifolia and Sesbania sesban. Bacterial Empire, 3(3), 20-24. https://doi.org/10.36547/be.2020.3.3.20-24

[10] Emitaro, W.O., Musyimi, D.M., Otiato, D.A. and Onyango, B. (2017). Morphological, Biochemical and Molecular Characterization of Xanthomonas campestris pv. vesicatoria on African nightshade (Solanum scabrum). International Journal of Biosciences, 11(1), 190-197.

[11] Emitaro, W.O., Musyimi, D.M., Otiato, D.A. and Buyela, D.K. (2018). Antibacterial effect of Euphorbia hirta and Bidens pilosa extracts against Xanthomonas campestris Pv vesicatoria isolated from diseased African nightshade (Solanum scabrum). International Journal of Microbiology and Mycology, 7 (5), 1-6.

[12] Gayathri, A. and Ramesh, K.V. (2013). Antifungal Activity of Euphorbia Hirta L . Inflorescence Extract against Aspergillus Flavus -- A Mode of Action Study. International Journal of Current Microbiology and Applied Science, 2(4), 31-37.

[13] Gracelin, D. H. S., John, A.D.E.B., Benjamin, P.J. and Rathna, K. (2012). Detection and Identification of Xanthomonas campestris pv.centellae on Leaves of Centella asiatica Collected in Tamilnadu. Asian Journal of Pharmaceutical and Clinical Research, 5(3), 11-13.

[14] Hossain, M.D.F., Hasan, S.M.Z., Zannati, F.Z., Habiba, F.M.H., Asadul, M.I. and Biswanath, S. (2018). Biological Control of Bacterial Leaf Spot Disease of Papaya (Carica papaya) Through Antagonistic Approaches using Medicinal Plants Extracts and Soil Bacteria," International Journal of Pure and Applied Biosciences, 6(1), 1-11. https://dx.doi.org/10.18782/2320-7051.6137.

[15] Izah, S.C. (2018). Some Determinant Factors of Antimicrobial Susceptibility Pattern of Plant Extracts. Research and Review Insight, 2(3), 1-4.

[16] Kebede, M., Amare, A. and Mohammed (2013). Efficacy of Plant Extracts , Traditional Materials and Antibacterial Chemicals Against Xanthomonas campestris pv . vesicatoria on Tomato Seed. African Journal of Microbiology Research, 7(20), 2395-2400.

[17] Le K.D., Kim. J., Yu, N.H., Kim, B., Lee, C.W. and Kim, J.C. (2020). Biological Control of Tomato Bacterial Wilt, Kimchi Cabbage Soft Rot, and Red Pepper Bacterial Leaf Spot Using Paenibacillus elgii JCK-5075. Frontiers in Plant Science, 11(775),1-12. https://doi.org/10.3389/fpls.2020.00775.

[18] Mamun, M. S. A. and Ahmed, M. (2011). Prospect Of Indigenous Plant Extracts In Tea Pest Management Indigenous Plant Extracts In Tea," International Journal of Agricultural Research Inovation and Technology, 1, 16–23.

[19] Mbega, E.R., Mortensen, C.N., Mabagala, R.B. and Wulff, E.G. (2012). The Effect of Plant Extracts as Seed Treatment to Control Bacterial Leaf Spot Of Tomato In Tanzania," Journal of General Plant Pathology, 78, 277-286.

[20] Nirosha, M., Pavani, K., Priya, A.K., Haseena, U. (2019). Jyothsna, S. Antimicrobial Activity of Organic Leaf Extract of Sesbania sesban Against Gram Negative Pathogenic Bacteria. International Journal of Life science and Pharmacy Research, 9(2), 30-38. http://dx.doi.org/10.22376.

[21] Ondieki, M. and Aguyoh, J. (2011). Variations in Growth and Yield Characteristics of Three Black Nightshade Species Grown under High Altitude Conditions. Agriculture and Biology Journal of North Americ, 2(3), 1-6.

[22] Opara, E.U. and Obani, F.T. (2010). Performance of Some Plant Extracts In the Control of Bacterial Spot Disease of Solanum. Agricultural journal, 5(2), 45-49.

[23] Perez, S.M., Biondi, E., Laurita, R., Proto, M., Sarti, F., Gherardi, M., Bertaccini, A., Colombo, V. (2019). Plasma Activated Water as Resistance Inducer Against Bacterial Leaf spot of Tomato. PLoS ONE, 14(5), 1-19. https://doi.org/10.1371/journal.pone.0217788

[24] Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J.D., Paret, M.L., Vallad, G.E. and Jones, J.B. (2015). Bacterial Spot of Tomato and Pepper: Diverse Xanthomonas Species with a Wide Variety of Virulence Factors Posing a Worldwide Challenge. Molecular Plant Pathology, 16(9), 907–920.

[25] Roach, R., Mann, R., Gambley, C.G., Shivas, R.G. and Rodoni, G. (2020). Pathogenicity and Copper Tolerance in Australian Xanthomonas species Associated with Bacterial Leaf spot. Crop Protection, 127, 1-11. https://doi.org/10.1016/j.cropro.2019.104923.

[26] Roach, R., Mann, R., Gambley, C.G., Shivas, R.G. and Rodoni, G. (2017). Identification of Xanthomonas Species Associated with Bacterial Leaf Spot of Tomato, Capsicum and Chilli Crops in Eastern Australia. European Journal of Plant Pathology, 150, 595-608. https://doi.org/10.1007/s10658-017-1303-9.

[27] Salhi, N., Sultan, A.M., Saghir, V.T., Iman, B.N.G. and Samia, B. (2017). Antifungal Activity of Aqueous Extracts of Some Dominant Algerian Medicinal Plants," BioMed Research International, 1- 6. https://doi.org/10.1155/2017/7526291.

[28] Shandukani, P.D., Shonisani, C.T., Peter, M. and Kgabo, M.M. (2018). Antibacterial Activity and in Situ Efficacy of Bidens pilosa Linn and Dichrostachys cinerea Wight et Arn Extracts Against Common Diarrhoea-Causing Waterborne Bacteria. BioMed Central Complementary and Alternative Medicine, 18(171), 1-10. https://doi.org/10.1186/s12906-018-2230-9.

[29] Syed-Ab-Rahman, S.F., Carvalhais, L.C. and Omar, D. (2020). Development of Plant-based Emulsion Formulations to Control Bacterial Leaf Blight and Sheath Brown Rot of Rice. Heliyon, 6, 1-6.

[30] Verma, A.K. and Agrawal K. (2017). In vitro Evaluation of Antibacterial Activity of Some Medicinal Plants against Xanthomonas pisi Causing Leaf Spot of Pea. International Journal of Pharmaceutical Sciences Review and Research, 45(2), 156-159