ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 12, pp. 22571-22575, December, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

LAWSONIA INERMIS LINN. : A PERSUASIVE AGENT TO CONTROL LEAF SPOT DISEASE OF MAIZE CAUSED BY *CURVULARIA LUNATA*

Tansukh Barupal and Kanika Sharma

Department of Botany, M.L.S. University, Udaipur-313001 Rajasthan, India

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1292

ARTICLE INFO

Article History:

Received 10th September, 2017
Received in revised form 14th October, 2017
Accepted 08th November, 2017
Published online 28th December, 2017

Key Words:

Successive extraction, Bioactive agent, Poison food technique, MIC, MFC, Phytotoxicity.

ABSTRACT

Lawsonia inermis extracts have been widely used for medicinal, cosmetic and preservative purposes. Natural resources have always provided good leads for the development of bioactive agents. In the context of the present study, antimicrobial activity of *Lawsonia inermis* extracts against fungus *Curvularia lunata* has been assessed. We tried six different solvents for successive extraction; the purpose was to screen out the best extract in term of its fungicidal action. Among all solvent extracts, acetone extract showed greatest percent (70.15%) inhibition of mycelia growth of target fungi. The commonly used laboratory method, poison food technique was used to evaluate and screen the *in vitro* antifungal activity. Mancozeb and bavistin were used as standards. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of acetone fraction of *Lawsonia inermis* Linn. were investigated against *Curvularia lunata* and phytotoxicity of best partially purified extract was observed. Our result shows that acetone fraction of *Lawsonia inermis* Linn. has maximum antifungal activity and can be used as a powerful fungicide against *Curvularia lunata* in treating leaf spot disease of maize.

INTRODUCTION

The leaf spot disease of maize is caused by *Curvularia lunata*, in tropical and subtropical regions across the globe (Bisht *et al.,* 2013). This disease is recognized as one of the most significant yield-limiting diseases of maize worldwide. The common symptoms include necrotic lesion surrounded by visible halo (Huang *et al*., 2005; Li *et al*., 2006). The disease cause significant damage especially in maize growing region (Huang *et al*., 2005; Akinbode, 2010). There are many chemically synthesized fungicides that can be used to control *Curvularia lunata*. Many important pathogens develop a resistance to synthetic fungicide due to its indiscriminate use (Boo and Gangwar, 2017). Besides this it has been proved that chemically synthesized fungicides cause serious environmental problems and also are poisonous to non-target organisms (Hellawell, 1986; Holmes and Eckert, 1999; Goetz and Dix, 2009; Ochoa-Acuna *et al*., 2009). So, use of plant based fungicides in the management of plant disease is gradually gaining importance due to their green and cost effective nature (Pal *et al*., 2013).

Lawsonia inermis is an evergreen shrub belongs to Family Lythraceae. It is well known for its medicinal properties (Alia *et al*., 1995; Ali *et al*., 2001; Habbal *et al*., 2005). Plant produces a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids. It has been establish that these product exhibit antimicrobial activity (Pavlidis, 1991; Habbal *et al*., 2005; El-Hag *et al*., 2007; González-Lamothe *et al*., 2009). Pharmacological evaluation of *Lawsonia inermis* to confirm its antimicrobial and anti-fungal properties has already been reported by many groups (Mollik *et al*., 2009; Hema *et al*., 2010; Borade *et al*., 2011). Many studies have already been done on the spot disease of paddy plant, but to the best of our knowledge, the similar reports on maize are absent. In this study, we investigated the effect of the *Lawsonia inermis* extracts on *Curvularia lunata*, which is the causative organism of the spot disease in maize.

MATERIAL AND METHOD

Poison food technique was used to evaluate or screen the *in vitro* antifungal activity of a *Lawsonia inermis* extracts. Six different solvents were used for extraction purpose to determine the best partially purified extract in term of maximum inhibitory effect. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was determined against *Curvularia lunata* for solvent extract showing maximum inhibitory effect.

Corresponding author:* **Tansukh Barupal
Department of Botany, M.L.S. University, Udaipur-313001 Rajasthan, India

Plant Material

The plants as a fresh leaf were harvested from Mohanlal Sukhadia University, Udaipur in India and identified as *Lawsonia inermis (*Voucher specimen number RUBL211447) at Department of Botany, University of Rajasthan, Jaipur, India. The leaves were collected and washed thoroughly with water and air dried under shade and then ground in an electrical grinder. The ground material was passed through a sieve (Mesh size 60) to obtain a fine powder which was used to prepare the extracts.

Collection of partially purified fraction using hot extraction method

Hot extraction method is Serial exhaustive method which used for successive separation of different phytochemical constituents present in dried plant material (Harborne, 1984).
Solvent series used for successive extraction was as follows:
Petroleum ether → Benzene → Chloroform → Acetone →Methanol →Water

In hot extraction method successive extraction was done using Soxhlet apparatus (Harborne, 1984). In this process use of different solvents ensure complete extraction of all of plant metabolites with respect to each solvent. Every time before extracting with next solvent the plant material is dried at temperature up to 50°C in an oven. 40 gm dry plant powder will be kept in Soxhlet extraction unit and extraction will be done separately 280 ml of each solvents.

The partially purified fraction was vacuum dried in a rotary evaporator. The fractions weighed and their percentage extractive value estimated by the following formula:

$$\text{Percent extractive value} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Assay of antifungal activity of partially purified fractions of Lawsonia inermis Linn. Leaf

Isolation, purification and identification of pathogen

Disease plant material was collected from the agriculture field of RCA, Udaipur. The isolation of pathogenic fungus was done by Agar plate method (Horn, 2005) further subculture was through from the periphery of mycelia growth and culture are being maintained on media i.e. Potato Dextrose Agar at 4^0C.
The culture was identified as fungus *Curvularia lunata* by the senior maize pathologist (AICRP-Maize New Delhi) Professor S. S. Sharma, Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and plant technology.

Antifungal activity of partially purified fraction of Lawsonia inermis Linn. leaf extracts

The antifungal activity of partially purified fractions of *Lawsonia inermis* leaf extract was tested using poison food technique (Grover and Moore, 1962). In respect 100 mg of extract was dissolved in 10 ml acetone to prepare stock solution of 10mg/ml concentration.

1 ml of stock solution was mixed with 9 ml molten sterile PDA culture medium and further this mixture was poured into pre-sterilized petri-plates (9 cm diameters) and allowed to solidify at room temperature. Thus prepared petri-plates were inoculated aseptically with 6 mm disc of test pathogen's cultures. The petri-plates were then incubated at 28±2 ^{0}C for seven days. Bavistin, mancozeb and only PDA culture media (water) are used as control series along with test samples. Antifungal activity of extract was measured as a function of increasing in growth of 6 mm disc of inoculums.

After seven day of incubation the Average diameter of the fungal colonies was measured and mycelial growth in percentage was calculated by the following formula given below:

$$\text{Mycelial growth inhibition} = \frac{gc - gt}{gc} \times 100$$

gc= growth of mycelia colony after 7days incubation period in control set subtracting the diameter of inoculums disc.
gt= growth of mycelia colony after 7days incubation period in treatment set subtracting the diameter of inoculums disc.

Minimum inhibitory concentration (MIC) determination:
MIC was determined by Broth dilution method against fungi (Collee *et al*., 1996). The extract was serially diluted with sterile distilled water up to the concentration of 20 mg/ml and then introduced into a test tube containing potato dextrose broth (PDB). The extract was added to 14 different test tubes in such a way that test tube no.1 contain highest 2000 µg/ml and test tube 14 contain the lowest concentration of extract 0.244 µg/ml (v/v).

Spore suspension 100 µl ($1x10^6$ spores per ml) of target fungi was inoculated in the test tube containing PDB medium and incubated for 9 days at 25± 2 C. The control tubes containing PDB medium were inoculated only with fungal suspension. Three replicates of each concentration were maintained.

Minimum fungicidal concentration (MFC) determination

Estimation of MFC of selected plant extract a loopful of fungal biomass taken from each test tubes containing MIC as well as higher concentration of extract and inoculated into tubes containing sterile PDA medium and incubated. Presence or absence of growth was observed after respective time. Appearance of growth indicated that the extract concentration was just fungistatic and and absence of growth indicated that extract concentration was fungicidal.

Phytotoxicity of partially purified acetone extract on seed germination of Maize

The percentage seed germination at different concentration (5 mg/ml; 10 mg/ml in acetone) of partially purified acetone extract was calculated using the Standard Blotter Method (SBM) established by International Seed Testing Association (ISTA). The randomly selected 2 set of 30 seeds were surface sterilized with 2% sodium hypochlorite for one minute and carefully washed 2-3 times with deionized water. After this, each set of seeds were treated with different concentration of selected extract for 30 minutes. Each set of treated seeds were plated at the rate of 10 seeds per plate. Moist blotter paper was placed in sterilized Petri plates to maintain requisite moisture subsequently seeds were placed on it and covered with a lid. Petri plates were incubated at 27±2^{0}C. There are also used one set of untreated seed used as water control. After one week, the

Petri plates were screened, and percentage seed germination was calculated using the following formula:

$$\text{Percentage seed germination} = \frac{\text{Total no. of seeds germinaed}}{\text{Total no. of seed plated}} \times 100$$

RESULTS

Extraction, purification, and identification of antibiotic activity

Extraction was done with six different solvents e.g. petroleum ether, benzene, chloroform acetone, methanol, and water. Percentage extractive values were in range from 4.47-18.63 (Table no.1) Methanol shows maximum percentage extractive value which is 18.63 and petroleum ether shows the minimum value of 4.47.

Table 1 Percent extractive value of different fraction of *Lawsonia inermis* leaf

S. No.	Solvent	Percent extractive value
1.	Petroleum ether	4.47
2.	Benzene	1.82
3.	Chloroform	1.22
4.	Acetone	9.77
5.	Methanol	18.63
6.	Water	4.5

In order to determine antifungal activity of partially purified extract of the *Lawsonia inermis* Linn was tested *in vitro* against *Curvularia lunata* growing on PDA agar in a Petri dish. The experimental results are shown in Fig. 1.

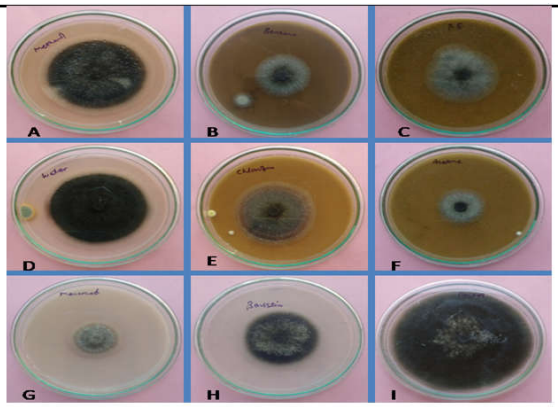

Fig:1. Petri dish photo of antifungal activity of partially purified fractions of *Lawsonia inermis* Linn. using poison food technique: (A) Methanol, (B) Benzene ,(C) Petroleum ether (D)Water,(E) Chloroform,(F)Acetone,(G) Mancozeb,(H)Bavistin,(I)Control.

After seven days of incubation, all the extract showed antifungal activity at varying percentage (Table 2).

Table 2 Antifungal activity of partially purified fractions of Lawsonia inermis leaf against *Curvularia lunata*

S.No.	Type of extract	Growth Diameter after 7 days (mm)	% Mycelial growth inhibition
1.	Petroleum ether	40.67±2.08	50.80
2.	Benzene	31.00±1.73	62.50
3.	Chloroform	48.33±0.57	41.53
4.	Acetone	24.67±0.57	70.15
5.	Methanol	65.00±1.00	21.37
6.	Water	64.67±0.57	21.33

Percent inhibition of mycelial growth was ranging from 21.33 to 70.15%. The highest percent inhibition of mycelial growth was recorded in case of acetone extract (70.15%), whereas the lowest was observed in water (21.33%) Among all tested extracts acetone fraction was found to be most potent one and was selected to determine MIC and MFC values.

In order to determine the Minimum inhibitory concentration (MIC) of acetone extract of *Lawsonia inermis*, *Curvulria lunata* spore suspension were treated with different concentrations of *Lawsonia* extract ranging from 20 to 0.0024 mg/ml using broth dilution method. The experiment results are shown in Fig. 2. After nine days of incubation, test tubes with concentration from 20- 0.625 mg/ml (1-6) showing no mycelium of colony whereas colonies were showing in a test tube with concentration 0.312 to 0.0024 mg/ml (7-14) and also in control.

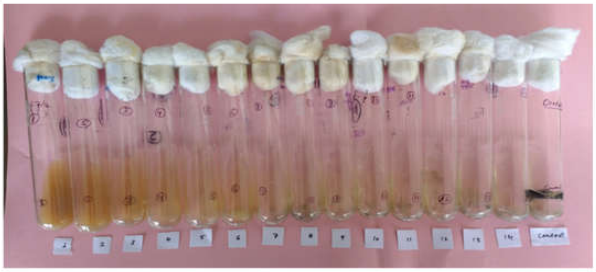

Fig. 2. Minimum Inhibitory Concentration (MIC) of acetone fraction observed in test tube number 6 at 62.5 µg/ml against *Curvularia lunata*.

Therefore, the minimum inhibitory concentration (MIC) of acetone extract of *Lawsonia* was determined to be 0.625 mg/ml (fig 2.) Minimum fungicidal concentration (MFC) was also determined, and it was observed at 1.25 mg/ml (fig 3). The percentage seed germination of 2 set of treated seed was observed 100 % and in control, it found 100%.

Table 3 Antifungal activity of standard fungicide with water control against *Curvularia lunata*

S.No.	Standard fungicides and water control	Growth Diameter after 7 days (mm)	% Mycelial growth inhibition
1.	Mancozeb	22.67±1.52	72.57
2.	Bavistin	52.67±1.52	36.28
3.	Water(control)	82.67±0.57	NI

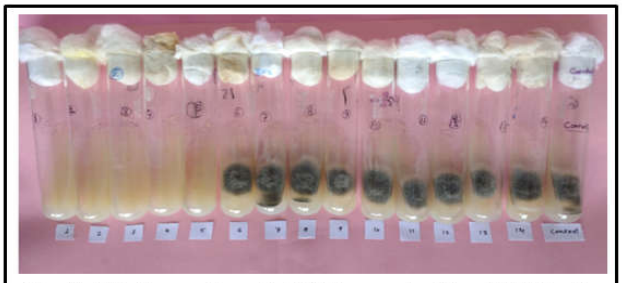

Fig. 3. Minimum Fungicidal Concentration (MFC) of acetone fraction observed in test tube number 5 at 125 µg/ml against *Curvularia lunata*.

DISCUSSION

Lawsonia inermis is a medicinally important plant. It has been shown to be effective against a variety of fungi, e.g., *Microsporum, Trichophyton and Candida albicans* (Hema *et al.,* 2010). During the antifungal screening, *Lawsonia inermis* leaves were found to exhibit strong fungicidal properties.

Lawsonia exhibited absolute toxicity (Rosenthal *et al.,* 2008) against ringworm. *Lawsonia* leaves are also used as a local anesthetic (Nirmalan and Baldwin, 1997), anti-inflammatory and for treating mouth ulcers (Alia *et al.,* 1995).

The present study was conducted to extract natural antifungal compounds from medicinal plants *Lawsonia inermis*, particularly to target fungi *Curvularia lunata*. Acetone extracts of *Lawsonia inermis* leaves showed maximum activity against *Curvulria lunata*. It is interesting to note that antifungal activity of some solvent extract was much higher than the tested control.

Quinones compounds are present in *Lawsonia inermis* plant (Pavlidis, 1991). They are an aromatic compound with two ketone substitutions. Quinones are a highly active compound with universal presence. The fungicidal action of *Lawsonia inermis* is due to the presence of naphthoquinones. (Holder and Boyce, 1994). *Lawsonia* leaves contain up to 5% by weight of the compound (2-hydroxy-1,4-naphthoquinone). The conversion between hydroquinone and quinone occurs easily through oxidation and reduction reactions. Oxidation reduction potential of the quinone-hydroquinone pair is very important in many biological processes. Quinones are a source of stable free radical in addition it also forms an irreversible complex with negative charge amino acid present in protein molecule (Tan and Berridge, 2008). This serves as the main reason for the potential antimicrobial effect of Quinones. Additionally, they also inhibit cell growth in culture medium (Del córdoba-pedregosa et al., 2006).

CONCLUSION

We concluded that the *Lawsonia inermis* Linn. shows significant antifungal activity against *Curvularia lunata* and further work is required to demonstrate its activity against other diseases cause by other fungi and same fungi in other plants as well, and further its antifungal activity can also be used to developed bio-formulation to control of leaf spot disease of maize in the way of eco-friendly.

Acknowledgement

This research work was supported by University Grant Commission, Delhi, India.

References

Akinbode, O.A. (2010): Evaluation of antifungal efficacy of some plant extracts on Curvularia lunata, the causal organism of maize leaf spot. *African J. Environ. Sci. Technol.* 4(11): 797–800.

Alia, B.H., Bashir, A.K., and Tanira, M.O.M. (1995): Anti-Inflammatory, Antipyretic, and Analgesic Effects of Lawsonia inermis L.(Henna) in Rats. *Pharmacology* 51(6): 356–363Available at http://dx.doi.org/10.1159/000139347.

Ali, N.A.A., Jülich, W.D., Kusnick, C. and Lindequist, U. (2001): Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J. Ethnopharmacol*. 74(2): 173–179.

Bisht, S., Kumar, P., Srinivasanraghavan, A. and Purohit. J. (2013): In vitro management of Curvularia leaf spot of maize using botanicals, essential oils and bio-control agents. *Bioscan Suppl. Med. Plants* 8: 731–733.

Boo, K. and Gangwar, M. (2017): Antifungal Activity of Endophytic Actinomyetes against Fusarium Wilt (Fusarium oxysporum) of Banana Trees (Musa acuminata). *Int. J. Curr. Microbiol. Appl. Sci.* 6(6): 328–337Available at http://dx.doi.org/10.20546/ijcmas.2017.606.039.

Borade, A.S., Kale, B.N. and Shete, R.V. (2011): A phytopharmacological review on Lawsonia inermis (Linn.). *Int J Pharm Life Sci* 2(1): 536–541.

Collee, F.G., Miles, R.S. and Watt, B. (1996): Test for identification of bacteria. In: *Mackie and McCartney Practical Medical Microbiology*, Longman Singapore publishers Lmd., Singapore, p. 131-150**.**

DEL CÓRDOBA-PEDREGOSA, M.C., Villalba, J.M., Gonzalez-Aragon, D., Bello, R.I. and Alcain, F.J. (2006): Cellular density and cell type are the key factors in growth inhibition induced by 2, 5bis [1-aziridinyl]-1, 4 benzoquinone (DZQ). Anticancer Res. 26(5A): 3535–3540.

El-Hag, A.G., Al-Jabri, A.A. and Habbal. O.A. (2007): Antimicrobial properties of Lawsonia inermis (henna): a review. *Aust. J. Med. Herbal*. 19(3): 114.

Goetz, A.K., and Dix, D.J. (2009): Mode of Action for Reproductive and Hepatic Toxicity Inferred from a Genomic Study of *Triazole Antifungals. Toxicol. Sci*. 110(2): 449-462Available at http://dx.doi.org/ 10.1093/toxsci/kfp098.

González-Lamothe, R., Mitchell, G. Gattuso, M.. Diarra, M.S., Malouin, F. and Bouarab, K. (2009): Plant antimicrobial agents and their effects on plant and human pathogens. *Int. J. Mol. Sci.* 10(8): 3400–3419.

Grover, R.K. and Moore, J.D. (1962): Toximetric Studies of Fungicides Against Brown Rot Organisms, Sclerotinia-Fructicola And S-LAXA. *Phytopathology* 52(9): 876-.

Habbal, O.A., Al-Jabri, A.A., El-Hag, A.H., Al-Mahrooqi, Z.H. and Al-Hashmi, N.A. (2005): In-vitro antimicrobial activity of Lawsonia inermis Linn (henna). A pilot study on the Omani henna. *Saudi Med. J.* 26(1): 69–72.

Harborne, J.B. (1984): Methods of Plant Analysis. *Phytochem. Methods*: 1–36Available at http://dx.doi.org/10.1007/978-94-009-5570-7_1.

Hellawell, J.M. (1986): Effects of Toxic Materials. Biol. Indic. Freshw. *Pollut. Environ. Manag.*: 212–328Available at http://dx.doi.org/10.1007/978-94-009-4315-5_7.

Hema, R., Kumaravel, S., Gomathi, S. and Sivasubramaniam, C. (2010): Gas chromatography-Mass Spectroscopic analysis of Lawsonia inermis leaves. *Life sci. Journal-Acta Zhengzhou Univ. Overseas* Ed. 7(4): 48–50.

Holder, I.A., and Boyce, S.T. (1994): Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns 20(5): 426-429Available at http://dx.doi.org/10.1016/0305-4179(94)90035-3.

Horn W.B. (2005): Colonization of wounded peanut seed by soil fungi selectivity for species from *Aspergillus* section *Flavi. Mycologia* 97: 202-217.

Holmes, G.J. and Eckert, J.W. (1999): Sensitivity of Penicillium digitatum and P. italicum to Postharvest Citrus Fungicides in California. *Phytopathology* 89(9): 716–721Available at http://dx.doi.org/10.1094/phyto.1999.89.9.716.

Huang, J., Zheng, L. and Hsiang. T. (2005): First report of leaf spot caused by Curvularia verruculosa on Cynodon sp. in Hubei, China. Plant Pathol. 54(2): 253Available at http://dx.doi.org/10.1111/j.1365-3059.2005.01126.x.

Li, Y., Xu, M. and Zou, X. (2006): Heterotrophic Soil Respiration in Relation to Environmental Factors and Microbial Biomass in Two Wet Tropical Forests. *Plant Soil* 281(1–2): 193–201Available at http://dx.doi.org/10.1007/s11104-005-4249-1.

Mollik, A.H., Azam, N.K., Ferdausi, D. Jahan, R. and Rahmatullah, M. (2009): A survey of medicinal plants used to treat cattle diseases in satkhira district, Bangladesh. *Planta Med.* 75(9) Available at http://dx.doi.org/10.1055/s-0029-1234533.

Nirmalan, M. and Baldwin. J. (1997): Anaesthetic implications of henna. *Eur. J. Anaesthesiol*. 14(6): 665–666Available at http://dx.doi.org/10.1097/00003643-199711000-00021.

Ochoa-Acuña, H.G., Bialkowski, W., Yale, G. and Hahn, L. (2009): Toxicity of soybean rust fungicides to freshwater algae and Daphnia magna. *Ecotoxicology* 18(4): 440–446Available at http://dx.doi.org/10.1007/s10646-009-0298-1.

Pal, G.K., Kumar, B. and Shahi, S.K (2013): Antifungal activity of some common weed extracts against phytopathogenic fungi Alternaria spp. *Int. J. Univers. Pharm. Life Sci.* 3(2): 6–14.

Pavlidis, V.H. (1991): Organic Chemistry (4th edition). R J Fessenden and J S Fessenden, Brooks/Cole, California, 1990. Pp 1137. £19.95. ISBN 0 534 98175 5. Appl. Organomet. Chem. 5(2): 139Available at http://dx.doi.org/10.1002/aoc.590050213.

Rosenthal, V.D., Maki, D.G., Mehta, A., Álvarez-Moreno, C., Leblebicioglu, H., Higuera, F., Cuellar, L.E., Madani, N., Mitrev, Z., Dueñas, L., Navoa-Ng, J.A., Garcell, H.G., Raka, L., Hidalgo, R.F., Medeiros, E.A., Kanj, S.S., Abubakar, S., Nercelles, P. and Pratesi, R.D. (2008): International Nosocomial Infection Control Consortium report, data summary for 2002-2007, issued January 2008. *Am. J. Infect. Control* 36(9): 627–637Available at http://dx.doi.org/10.1016/j.ajic.2008.03.003.

Tan, A.S. and Berridge, M. V. (2008): Differential effects of redox-cycling and arylating quinones on trans-plasma membrane electron transport. *BioFactors* 34(3): 183–190Available at http://dx.doi.org/10.1002/biof.5520340302.

How to cite this article:

Tansukh Barupal and Kanika Sharma.2017, Lawsonia Inermis Linn. : A Persuasive Agent To Control Leaf Spot Disease of Maize Caused By Curvularia lunata. *Int J Recent Sci Res.* 8(12), pp. 22571-22575.
DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1292

****