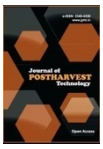

Journal of Postharvest Technology
2022, **10**(3): 94-101
www.jpht.in

RESEARCH ARTICLE

In vitro antifungal activity of various elicitors and binders against *Alternaria solani*

Bhanu Raj Meena[1]*, Deepali Chittora[2], Jyoti Yadav[2], Sanjeev Meena[2], Suresh Kumar[1], Tripta Jain[2], Kanika Sharma[2]

[1]Ramjas College, University of Delhi, 110007
[2]Microbial Research Laboratory, Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur, India

Received: 11.06.2022 Accepted: 28.07.2022

ABSTRACT

In the present study, *in vitro* antifungal activity of various elicitors like ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, coconut oil cake and neem oil cake and binders like cow dung, guar gum and gum acacia were used with the aim to study on antifungal activity. Plant products/extracts have been observed to be successful over traditional fungicides and microbial biocontrol agents. Acacia gum has long been used in everyday applications and in traditional medicine. Cow dung also well-known as cow pad, is a constituent having crude protein, cellulose, hemicellulose and minerals. It is proficient organic manure employed to enhance plant yield in fields. 20gm elicitors and binders were dissolved in 100 ml of autoclaved water for 24 h. The mixture was then filtered and used for antifungal activity. Among the all Elicitors and all binders best optimum activity was observed for neem oil cake and cow dung i.e. 60.32% and 36.36 % respectively against *Alternaria solani*. Results suggested that best elicitor i.e. neem oil cake and binder cow dung cab be used to develop the plant extract based bio-formulation for effective control of early blight of potato in an eco- friendly manner. These elicitors and binders improve fertility of the soil in which plants or crops are planted. Thus, use of these components cut the additional cost of adding extra nutrients to soil. Neem cakes and cow dung can also get better the organic constituents of the soil by providing plenty of micro and macro nutrients.

Keywords: Bio-formulation, elicitors, binders, oil cakes, cow dung

Citation: Meena, B.R., Chittora, D., Yadav, J., Meena, S., Kumar, S., Jain, T. and Sharma, K. 2022. In vitro antifungal activity of various elicitors and binders against *Alternaria solani*. *Journal of Postharvest Technology*, **10**(3): 94-101.

INTRODUCTION

An investigation of alternate mechanism for the management of pests and pathogens is very necessary to control environmental pollution due to use of chemical (Kuc, 1987; Lyon et al., 1995). Current trends of utilization of plant based and natural products insist the substitution of synthetic additives with natural ones. Enormous array of secondary metabolites are produced by plants and this chemical diversity protects the plant from plant pathogens that cause harsh economic losses (Ghosh et al., 2013). Plant products/extracts have been observed to be successful over traditional fungicides and microbial biocontrol agents, for a broad

* *For correspondence*: B. R. Meena (Email: bhanurajmeena@gmail.com)

ISSN: 2348-4330

variety of pathogens (Amadioha 2000; Bowers and Locke 2004). In addition, herbal bio-formulations based on plant extract, are systemic, precise in action, nonphytotoxic, cost effective and have poor environmental retention (Saini, 2019).

Herbal formulations are balanced composition of combination of suitable elicitors and effective binders with plant extracts/plant parts. These natural plant based formulations have a lot of merits over synthetic ones as they are chemically inert, nontoxic, less expensive, biodegradable and extensively accessible (Choudhary and Pawar, 2014).

Addition of elicitors improves the effect of naturally found phytochemicals in selected plant extract. Elicitors have property to elicit the plant growth by providing some essential nutrients and in combination with plant extract, elicitors strengthen the plant or crop to fight with fungal pathogens (Juveriya and Ahmmed, 2016). For commercial use, it is necessary that the formulation is available at an affordable price. To reduce the cost of developed formulation use of appropriate and cheap elicitors and binders is a prime requirement. Oil cakes like neem oil cake are some easily available elicitors usually individually incorporated in any formulation.

Another important content that needs to be added in bio-formulation is a binder. Some naturally available binders usually incorporated in herbal formulation are Guar gum, gum acacia and cow dung. These are the natural organic fertilizers with high nitrogen content, and left over parts after oil extraction process. Guar gum, cow dung and gum *acacia* are most commonly used binders usually incorporated to bio-formulation.

Acacia gum has long been used in everyday applications and in traditional medicine (Lemenith and Teketay, 2003).Among these binders, cow dung also well-known as cow pad, is a constituent having crude protein, cellulose, hemicellulose and minerals. It is proficient organic manure employed to enhance plant yield in fields. Cow dung slurry is also used by people of our country for plastering the floors and walls of their houses. In literature, cow dung has been described as antimicrobial agent (Sushmita et al., 2014).

Therefore, in this study the antifungal activity of some selected binders and elicitors was assayed so that effective herbal formulation can be prepared against test pathogen.

MATERIALS AND METHODS

In the present study antifungal efficacy of a variety of elicitors and binders was observed against *Alternaria solani. In vitro* antifungal activity of six types of elicitors *i.e.* ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, coconut oil cake and neem oil cake and three type of binders *i.e.* guar gum ,gum acacia and cow dung were assayed.

Antifungal activity of various elicitors and binders against *Alternaria solani*

20 gm of elicitors like ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, coconut oil cake and neem oil cake and binders like guar gum, gum acacia and cow dung was dissolved in 100 ml of autoclaved water for 24 h. The mixture was then filtered and the filtrate was further used for antifungal efficacy. The antifungal efficacy of each elicitor was checked using poison food technique.

1 ml of each elicitor and binder was mixed with 9 ml molten sterile PDA culture medium was poured into pre-sterilized petri-plates (9 cm diameters) and allowed to solidify at room temperature. Accordingly prepared petri-plates were inoculated

aseptically with 6mm disc of test pathogen's cultures which was positioned at the centre of the plate. These inoculated petri-plates were then incubated at 28±2C for seven days, uninoculated PDA culture media were used as control series. Antifungal activity of each elicitor was measured as a function of increase in growth diameter of 6 mm disc of inoculums.

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

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

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

RESULTS

Table 1 and 2 depict the results of antifungal activity of elicitors and binders against test fungus. It was observed that among the elicitors included in these study maximum percent mycelial inhibition was observed with neem oil cake *i.e.* 60.32% followed by mustard oil cake and groundnut oil cake *i.e.* 49.16% and 49.16% respectively (Fig 1 A). In case of binders, maximum inhibition was observed with cow dung *i.e.* 36.36% while least was observed for gum acacia *i.e.* 25.20% (Fig. 1 B).

Table 1: Antifungal activity of various Elicitors against *Alternaria solani*

Elicitors	Growth diameter after 7 days (mm) ± SD	% mycelial growth inhibition
Neem oil cake	32±1.00	60.32
Mustard oil cake	41±1.00	49.16
Cotton oil cake	43±1.00	46.68
Sesame oil cake	47±1.00	41.73
Ground nut oil cake	41±1.00	49.16
Coconut oil cake	45±1.00	44.21
Control	80.66±0.577	--

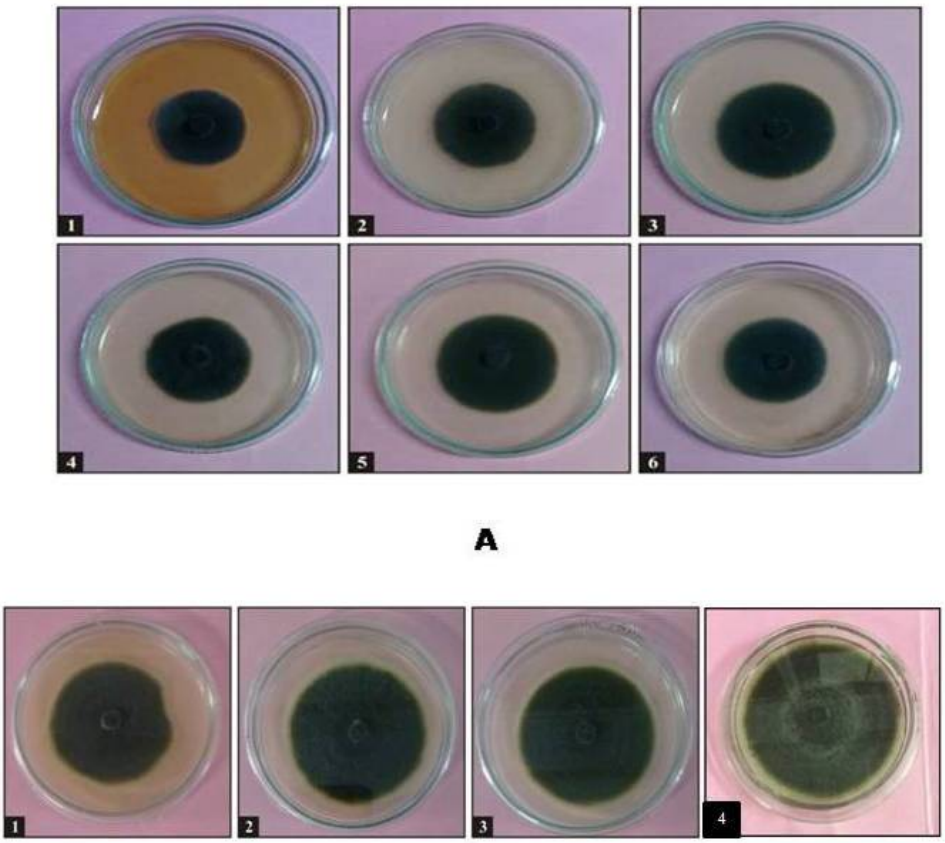

Figure 1: Antifungal activity of Elicitors and Binders.

(A) Elicitors (1) Neem oil cake (2) Mustard oil cake (3) Cotton oil cake (4) Sesame oil cake (5) Ground nut oil cake (6) Coconut oil cake

(B) Binders (1) Cow dung (2) Guar gum (3) Gum acacia (4) Control (Only PDA media)

(C)

Table 2: Antifungal activity of various Binders against *Alternaria solani*

Binders	Growth Diameter after 7 days (mm) ±SD	% mycelial growth inhibition
Cow dung	51.33±0.57	36.36
Guar gum	57.33±0.57	28.92
Gum acacia	60.33±0.57	25.20
Control	80.66±0.57	-

DISCUSSION

To protect and set up an agricultural scheme with ecological sustainability, it is very important to explore biological methods to not only manage the fungal infections in crop and plants, but also to sure productivity, quality of plants and to reduce environmental pollution. Herbal formulations formulated by combination of elicitors, binders and plant extract are one of the best ecofriendly approach to control fungal disease. Elicitors and binders complement the efficacy of plant extract which alone can be very expensive. Currently, binders are incorporated in new dosage forms to accomplish precise functions and in a few cases they openly or ultimately affect the extent and/or rate of release and action of plant extract (Atanasov et al., 2015).

Natural elicitors like ground nut oil cake, mustard oil cake, cotton oil cake, sesame oil cake, coconut oil cake, and neem oil cake and binders like cow dung, guar gum and gum acacia have been studied for antifungal activity (Hada and Sharma, 2016.). The marketable value of oil cakes relies on water and fat content found. Moisture typically accounts for between 20 and 30 percent of the weight, whereas the fat portion varies from a minimum of about 2.5 percent of the weight to 10–12 percent and in a number of cases even further, depending on the extraction procedure employed. Additionally, it certain as stock feed for animals, the residue can also be utilized as a fertilizer, after it has been appropriately steeped to avoid fermentation, which would injure the plants (Al-Momany and Al-Saket, 1989). Among the oil cakes under study, neem oil cake was found to be efficiently inhibiting the mycelial growth of *A. solani*.

Neem cake is the remaining part of the procedure to squash the neem seeds to get oil and it is known as a useful source of organic fertilizer. This type of fertilizer not only is good source of nutrition for plants but can also be utilized as inhibitors to manage several species of pathogenic fungi (Shah et al. 2008; Singh and Vyas 1984; Tiyagi and Alam 1995). Antimicrobial activity of neem oil cake might be due to the presence of azadirachtins and salannin (Del Serrone and Nicoletti, 2013).

Binders like guar gum and gum acacia are natural plant products and complement the antifungal efficacy of plant extract. Mostly nontoxic for human system, they show limited field persistence and have no residual threats. Cow dung is typically consist of organic matter together with fibrous material that release after passing of food through the cow's digestive system, among other liquid digest that has been left after the fermentation, absorption and filtration, then acidified, than absorbed again. Chemical composition of cow dung involves presence of C, N, H, O, P, etc. with salts, cells sloughed off as the digest went through the digestive tract, a little urea, mucus, in addition to cellulose, lignin and hemicellulose (Rajeshwari et al., 2016).

In present investigation cow dung was observed to be best binder that causes maximum inhibition of test fungus. It is very effective organic manure utilized to enhance improvement in plant yield in fields. Cow manure consists of three of the most essential nutrients *i.e.* N, P and K that plants require for their healthy growth. Whereas not all cow dung consists of the precise similar ratio of these minerals, research shows that cow dung has approximately about 3% N, 2% P and 1% K and the finest fraction is that the beneficial bacteria in cow dung leads to conversion of these essential nutrients into forms that are simply absorbed by plant roots. These nutrients are slowly infused into the soil allowing the plants to enjoy the benefits over longer periods (Mangtu Ram, 2017).

A study had been done by (Sushmita et al., 2014) to assess antibacterial and antifungal characteristics of cow dung extract in distil water, ethanol and n-hexane against *Candida*, *E. coli*, *Pseudomonas* and *Staphylococcus aureus* and observed it extremely efficient against these microbes. Cow dung was observed to suppress mycelia growth of plant pathogenic fungi like *Fusarium solani, Fusarium oxysporum* and *Sclerotinia sclerotiorum* (Basak et al., 2002). Spraying of cow dung extract was also

efficient for the controlling bacterial blight disease of rice and was uniformly efficient as *Pausthamycin*, *Penicillin* and *Streptomycin* (Mary et al., 2006).

Antagonistic properties of bacterial genera identified (*Bacillus* and *Pseudomonas)* from cow dung are well known. These research findings support the use of cow dung as a purifier in religious practices and for disease suppression in organic farming over the years (Sinha et al., 2009).

In addition to this, cow dung also plays significant role in plant healthy growth which might be due to the plenty of supply of organic matter, N, P and other nutrients which positively enhance the soil texture and structure. Cow dung is reported to boost the yields of cereals, legumes, oilseeds, vegetables and pastures and in improving the level of plant nutrient in soil, especially N, P and K (Swain and Ray, 2009).

Thus, the results obtained suggested that cow dung as a binder and neem oil cake as elicitors show best inhibitory activity against test fungus *Alternaria solani*.

CONCLUSION

Use of natural binders and elicitors is comparatively cost efficient since the fertilizer in them lasts longer. These elicitors and binders remain efficient until the next crop is planted as of its long sustaining organic fertilizer compounds too. Thus, use of these components cut the additional cost of adding extra nutrients to soil. Neem cakes and cow dung can also get better the organic constituents of the soil by providing plenty of micro and macro nutrients. These elicitors and binders improve fertility of the soil in which plants or crops are planted.

REFERENCES

Al-Momany, A. A., and I. Al-Saket. 1989. Effect of Endomycorrhizal Fungi on Maximizing the Efficiency of Olive Cakes as Fertilizer for Young Olives. Agricultural Sciences Series, 13, 31-42.

Amadioha, A. C. 2000. Controlling rice blast in vitro and in vivo with extracts of Azadirachta indica. Crop Protection, 19(5), 287-290.

Atanasov, Atanas G., Birgit Waltenberger, Eva-Maria Pferschy-Wenzig, Thomas Linder, Christoph Wawrosch, Pavel Uhrin, Veronika Temml, Limei Wang, Stefan Schwaiger, Elke H. Heiss, and others. 2015. Discovery and Resupply of Pharmacologically Active Plant-Derived Natural Products: A Review. Biotechnology Advances 33(8), 1582–1614.

Basak, A. B., Lee, M. W., and Lee, T. S. 2002. Inhibitive activity of cow urine and cow dung against Sclerotinia sclerotiorum of cucumber. Mycobiology, 30(3), 175-179.

Bowers, J. H., and Locke, J. C. 2004. Effect of formulated plant extracts and oils on population density of Phytophthora nicotianae in soil and control of Phytophthora blight in the greenhouse. Plant disease, 88(1), 11-16.

Choudhary, P. D., and Pawar, H. A. 2014. Recently investigated natural gums and mucilages as pharmaceutical excipients: an overview. Journal of pharmaceutics, 1-9.

Dean, R. A., and Kuć, J. 1987. Rapid lignification in response to wounding and infection as a mechanism for induced systemic protection in cucumber. Physiological and Molecular Plant Pathology, 31(1), 69-81.

Del Serrone, P., and Nicoletti, M. 2013. Antimicrobial activity of a neem cake extract in a broth model meat system. International journal of environmental research and public health, 10(8), 3282-3295.

Hada, Deepa., and Sharma, Kanika 2016. Comparative analysis of different elicitors and binders and development of herbal formulation containing Cassia fistula L. fruit pulp against Alternaria solani. International Journal of Agricultural Science and Research, 6, 371-380.

Juveriya, H. and A. Ahmmed 2016. A comparative study on the effects of three different organic manures (cow dung, vermicompost and neem cake) on the growth of Codiaeum variegatum. 35(4), 791-795.

Lemenith, M., and Teketay, D. 2003. Frankincense and myrrh resources of Ethiopia: II. Medicinal and industrial uses. SINET: Ethiopian Journal of Science, 26(2), 161-172.

Lyon, G. D., Reglinski, T., and Newton, A. C. 1995. Novel disease control compounds: the potential to 'immunize'plants against infection. Plant pathology, 44(3), 407-427.

Mary, C. A., Nair, S. K., and Saraswathy, P. 2006. Efficacy of prophylactic and curative sprayings on the control of bacterial blight of rice. Journal of Tropical Agriculture, 39(1), 73-75.

Paul, I., Bhattacharya, S., Chatterjee, A., and Ghosh, M. K. 2013. Current understanding on EGFR and Wnt/β-catenin signaling in glioma and their possible crosstalk. Genes and cancer, 4(11-12), 427-446.

Rajeshwari, S., E. Poongothai, and N. Hemalatha. 2016. Antimic Robial Activity of Cow Dung Extracts against Human Pathogens. Int. J of Current Pharmaceutical Research, 8(4), 9-12.

Ram, A. A. M. 2017. Panchagavya is a bio–fertilizer in organic farming. International Journal of Advanced Science Research, 2(5), 54-57.

Saini, N., Thakur, A., Kaur, P., and Gahlawat, S. K. 2019. Herbonanoceuticals: A Novel Beginning in Drug Discovery and Therapeutics. In Nanobiotechnology in Bioformulations. Springer, Cham, 161-180.

Shah, Farooq A., M. Gaffney, M. A. Ansari, M. Prasad, and T. M. Butt 2008**.** Neem Seed Cake Enhances the Efficacy of the Insect Pathogenic Fungus Metarhizium Anisopliae for the Control of Black Vine Weevil, Otiorhynuchs Sulcatus (Coleoptera: Curculionidae). Biological Control, 44(1), 111–15.

Singh, R., and K. M. Vyas. 1984. Influence of Certain Oil Cakes on Betelvine Phytophthora. Indian Phytopathology, 37(2), 359-361.

Sinha, R. K., Herat, S., Chauhan, K., and Valani, D. 2009. Earthworms vermicompost: a powerful crop nutrient over the conventional compost and protective soil conditioner against the destructive chemical fertilizers for food safety and security. Journal of Agriculture and Environmental Science, 5, 1-55.

Sushmita, S., Mishra A., and Pal A. 2014. Cow Dung-A Boon for Antimicrobial Activity. Life sciences Leaflets, 55, 60–63.

Swain, M. R., and R. C. Ray. 2009. Biocontrol and Other Beneficial Activities of Bacillus Subtilis Isolated from Cowdung Microflora. Microbiological Research, 164(2), 121–30.

Tiyagi, S. A., and Alam, M.M. 1995. Efficacy of Oil-Seed Cakes against Plant-Parasitic Nematodes and Soil-Inhabiting Fungi on Mungbean and Chickpea. Bioresource Technology, 51(2–3), 233–39.