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EC PHARMACOLOGY AND TOXICOLOGY
Research Article

Evaluation of Antifungal Potential of Elicitors and Binders

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Received: June 10, 2021; **Published:** November 30, 2021

Abstract

In the present study antifungal activity of elicitors like neem, mustard, coconut and *Pongamia* oilcakes and binders like cowdung, guar gum and gum acacia was assayed against *Alternaria solani* to find out antifungal potential. Various concentrations of elicitors i.e. 10% to 100% were used for antifungal assay. Initial screening of binders for antifungal activity against *A. solani* was done at 200 mg/ml to 0.39 mg/ml concentrations due to undissolving nature of gums at higher concentrations. Based on the results the substance giving best activity will be chosen for preparation of bioformulation. Best inhibitory activity was observed with 100% neem oil cake and percent mycelia growth inhibition was 51.32% against *Alternaria solani*. Initial screening of binders like cow dung, guar gum and gum *Acacia* (binders) for antifungal activity was done at 200 mg/ml to 0.39 mg/ml concentration due to dissolving nature of guar gum and gum *Acacia*. Among all binders assayed maximum inhibition of *A. solani* was observed with cow dung at 200 mg/ml concentration i.e. 36.87%. Second highest inhibition observed was 34.56% with gum *Acacia* at 200 mg/ml and 31.79% inhibition was observed with guar gum at 200 mg/ml. In the selection process of binder, cow dung showed maximum inhibition so 10% to 100% solutions of the cow dung was prepared and assayed for antifungal activity against *A. solani*. Maximum inhibition was observed with 100% solution of cow dung i.e. 41.74%. Water as control was maintained in which no elicitors, binders and fungicides used.

Keywords: *Elicitor; Binder; Antifungal activity; Fungicides; Secondary Metabolites*

Introduction

Plants are the richest source of secondary metabolites which are biologically active molecules and can be an alternative of agrochemicals. Plant products or plant based biological agents directly attack on pathogen without damaging the surrounding environment or induce resistance in plants. This is called eco-friendly systems in which there is no harm of animals humans or complete ecosystem [1,2].

In order to develop a new strategy as biological agent for control of plant pathogenic fungi, it is essential to find out active ingredients of biocontrol agents as elicitor and binder which promote the potential of plant extract to control the plant disease. Selection criteria of suitable elicitor and binder are totally based on the best antifungal activity against test fungi.

The term elicitor was used for molecules that are capable of inducing the production of phytoalexins, but it is now normally used for all compounds that stimulate any kind of plant defence [3,4]. Molecules of elicitor can attach to specific receptor proteins located on plant cell membranes [5]. Through Octadecanoid pathway intracellular defence signalling happens and receptors are recognized the molecular patterns of elicitors and this activity enhanced synthesis of metabolites which decrease damage and increase resistance to pests, disease or environmental stress [6].

Elicitors are of two types one is originating from pathogen called exogenous elicitors and another is endogenous elicitors originated from plants [7]. Elicitors can also be arranged as biotic or abiotic, physical or chemical, and complex or depending on their origin and

molecular structure. Among plant based elicitors, oil cakes are very common. These are residues of various plants like neem, mustard, peanut, sesame, coconut and *Pongamia* seeds etc. left behind after oil extraction. The composition of these oil cakes varies depending on the plant, growing condition and extraction methods. Antifungal activity of various oilcakes has been studied by several workers [8].

Binder is a substance which holds other materials together (elicitors and extracts) to form a smooth formulation. Generally, materials labelled as binders in various proportions or uses can have their roles reversed with what they are binding. Many types of binders are available in the market, some of which are natural and others are synthetic in nature. Natural binders commonly used are Cow dung, guar gum and gum *Acacia* etc [9,10].

Guar gum, also known as guaran, is the ground endosperm of guar beans. Guar gum powder is extracted from the guar seeds after a multistage industrial process. The guar seeds are dehusked, milled and screened to get the guar gum. It is produced as a free-flowing, off-white powder. The color of guar gum powder is whitish and yellowish having slight odour. Guar gum and its derivatives are widely used as binders and disintegrating agents in tablet dosage form [11].

Acacia gum has long been used in everyday applications and in traditional medicine. *Acacia* is a genus of shrubs and trees belonging to the subfamily Mimosoideae, of the family fabaceae or Leguminosae [12]. It is the exudate produced by the stem of *Acacia nilotica* (Babbul) also known as gum *Arabica* or Indian gum. It is used widely in the cooking industry to give body and texture to processed food products, in pharmaceutical industry as a demulcent and for drug delivery since it is readily available, is cost effective, eco-friendly, is capable of a multitude of modifications, is potentially degradable as well as compatible due to its natural origin [13,14].

Cow dung is the waste excreted by cows which consists of undigested residues of consumed matter which has passed through the gastrointestinal system of the animal. The undigested plant material that is rich in nutrients, micro-organisms, and their by products, 80% water are the major components of cow dung [15]. Cow dung has been observed to suppress mycelial growth of plant pathogenic fungi like *Fusarium solani, F. oxysporum* and *Sclerotinia sclerotiorum* [16].

Selection of suitable elicitor and binder is extremely important as they may show specific interaction with specific molecules. In the present study antifungal activity of elicitors like neem, mustard, coconut and *Pongamia* oilcakes and binders like cowdung, guar gum and gum acacia was assayed against *Alternaria solani* to find out the best possible combination for developing the bioformulation. Various concentrations of elicitors i.e. 10% to 100% were used for antifungal assay. Initial screening of binders for antifungal activity against *A. solani* was done at 200 mg/ml to 0.39 mg/ml concentrations due to undissolving nature of gums at higher concentrations. Based on the results the substance giving best activity was chosen for preparation of bioformulation.

Materials and Methods

Assay of antifungal activity of elicitors and binders

The inhibitory activity of neem oilcake, mustard oilcake, coconut oilcake and *Pongamia* oilcake against *Alternaria solani* was tested using poison food technique [17]. 10% to 100% solution of 20 gm of all oilcakes were sued for antifungal activity. Solution of oilcakes were prepared by suspended the oilcake powder in autoclaved water for 24 hrs. After 24 hrs the extract were filtered and the fine solution were used for the preparation of various concentrations and assayed for antifungal activity (Figure 1). In order to select suitable and most efficient binder, initial screening of antifungal activity of guar gum, gum *Acacia* and cow dung individually was done at 200 mg/ml to 0.39 mg/ml concentrations. Each binder was dissolved into water to get clear solution and tested for antifungal activity. The substance showing best inhibitory activity was chosen for further experimental work (Maximum inhibition was recorded as lesser growth of fungi) (Figure 2). Percent mycelia growth inhibition was calculated by the following formula given below:

% Mycelial growth inhibition = gc-gt/gc×100.

Figure 1: *Crude extract of elicitors and binders.*

A: Elicitors

1. Neem oil cake (NOC)

2. Mustard oil cake (MOC)

3. Coconut oil cake (COC)

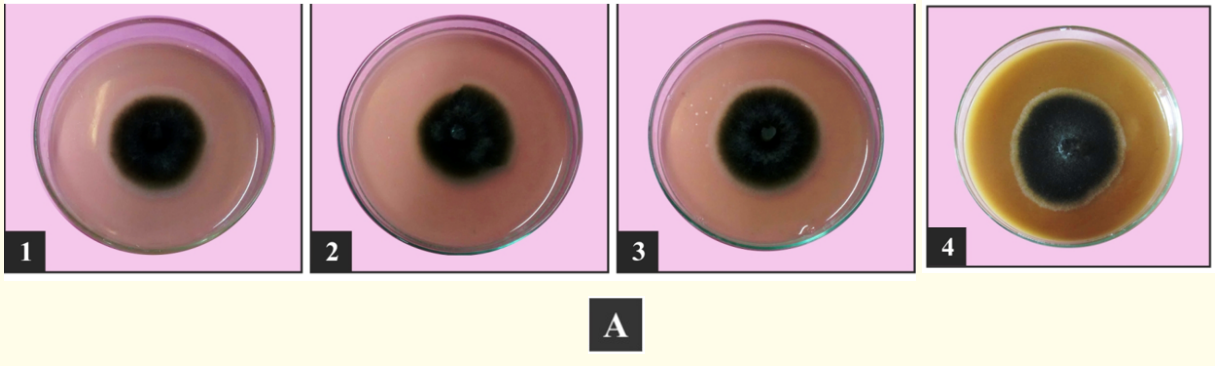
4. Pongamia oil cake (POC)

B: Binders

1. Cow dung

2. Gum Acacia

3. Guar gum.



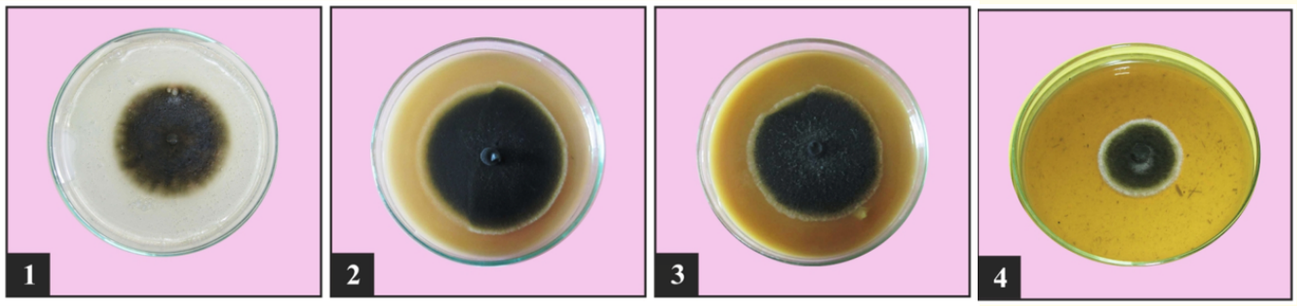


Figure 2: *Antifungal activity of elicitors and binders against Alternaria solani.*

A: Elicitors

1. 100% neem oil cake

2. 100% Mustard oil cake

3. 100% Coconut oil cake

4. 100% Pongamia Oil cake

B: Binders

1. 200 mg/ml Cow dung

2. 200 mg/ml Gum Acacia

3. 200 mg/ml Guar gum

4. 100% Cow dung.

Statistical analysis

All experiments were performed in triplicates (n = 3) and the data were presented as the mean ± standard deviation.

Results and Observations

Results suggested that antifungal activity increased with increasing concentration of elicitors and binders. Maximum activity was observed with 100% concentration. Hence this concentration was used for the preparation of bioformulation.

Results of antifungal activity of neem, mustard, coconut and *Pongamia* oilcake (elicitors) are present in table 1-8. 10% to 100% solutions of neem mustard, coconut and *Pongamia* oil cakes were assayed for antifungal activity against *Alternaria solani*. Best antifungal activity was observed with 100% neem oil cake and percent mycelia growth inhibition was 51.32% (Figure 3). The second highest inhibition was shown by 100% *Pongamia* oil cake *i.e.* 39.16%, 100% mustard oil cake showed 38.84% and 100% coconut oilcake showed 30.55% inhibition of *Alternaria solani* (Table 1-4 and figure 2A).

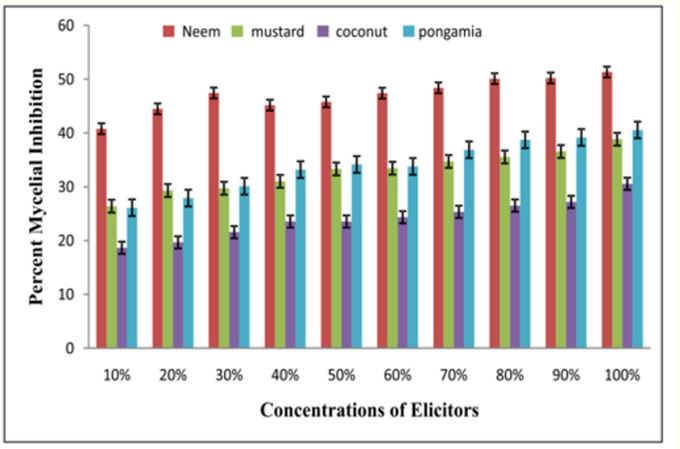


Figure 3: *Antifungal activity of elicitors against Alternaria solani.4. 100% Cow dung.*

S. No.	Neem oil cake	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	10%	42.83 ± 0.28	40.78
2.	20%	40.16 ± 0.37	44.47
3.	30%	40.03 ± 0.49	47.42
4.	40%	39.66 ± 0.41	45.16
5.	50%	39.23 ± 0.57	45.76
6.	60%	38.06 ± 0.57	47.38
7.	70%	37.33 ± 0.57	48.38
8.	80%	36.10 ± 0.20	50.08
9.	90%	36.02 ± 0.41	50.20
10.	100%	35.21 ± 0.30	51.32
11.	Control	72.33 ± 0.51	

Table 1: *Antifungal activity of neem oil cake against Alternaria solani.*

S. No.	Mustard oil cake	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	10%	53.23 ± 0.40	26.40
2.	20%	51.13 ± 0.55	29.31
3.	30%	50.83 ± 0.66	29.72
4.	40%	49.9 ± 0.45	31.01
5.	50%	48.23 ± 0.57	33.31
6.	60%	48.13 ± 0.47	33.45
7.	70%	47.23 ± 0.43	34.70
8.	80%	46.63 ± 0.41	35.53
9.	90%	45.89 ± 0.30	36.55
10.	100%	44.23 ± 0.30	38.84
11.	Control	72.33 ± 0.51	

Table 2: *Antifungal activity of mustard oil cake against Alternaria solani.*

S. No.	Coconut oil cake	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	10%	58.83 ± 0.32	18.66
2.	20%	58.1 ± 0.55	19.67
3.	30%	56.73 ± 0.45	21.56
4.	40%	55.73 ± 0.41	23.54
5.	50%	55.3 ± 0.45	23.54
6.	60%	54.73 ± 0.41	24.33
7.	70%	54 ± 0.26	25.34
8.	80%	53.16 ± 0.49	26.50
9.	90%	52.66 ± 0.30	27.19
10.	100%	50.23 ± 0.41	30.55
11.	Control	72.33 ± 0.51	

Table 3: *Antifungal activity of coconut oil cake against Alternaria solani.*

S. No.	Coconut oil cake	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	10%	53.45 ± 0.73	26.10
2.	20%	52.15 ± 0.32	27.89
3.	30%	50.56 ± 0.55	30.09
4.	40%	48.33 ± 0.52	33.178
5.	50%	47.63 ± 0.23	34.14
6.	60%	47.89 ± 0.21	33.78
7.	70%	45.66 ± 0.51	36.87
8.	80%	44.33 ± 0.57	38.71
9.	90%	44.00 ± 0.57	39.16
10.	100%	43 ± 0.57	40.55
11.	Control	72.33 ± 0.51	

Table 4: *Antifungal activity of Pongamia oil cake against Alternaria solani.*

Initial screening of binders like cow dung, guar gum and gum *Acacia* (binders) for antifungal activity against *Alternaria solani* was done at 200 mg/ml to 0.39 mg/ml concentration due to dissolving nature of guar gum and gum *Acacia*. Results are presented in table 5-7 and figure 2B. Among all binders assayed maximum inhibition of *A. solani* was observed with cow dung at 200 mg/ml concentration i.e. 36.87%. Second highest inhibition observed was 34.56% with gum *Acacia* at 200 mg/ml and 31.79% inhibition of *A. solani* was observed with guar gum at 200 mg/ml (Figure 4). In the selection process of binder for preparation of bioformulation, cow dung showed maximum inhibition so 10% to 100% solutions of the cow dung was prepared and assayed for antifungal activity against *A. solani*. Maximum inhibition was observed with 100% solution of cow dung i.e. 41.74% (Table 8 and figure 2B4). All data were compared with water as control in which no elicitors, binders and fungicides used (Table 9). Bavistin and Mancozeb were used as standard fungicides. Among them maximum inhibition of *A. solani* was recorded with mancozeb that was 78.80%.

S. No.	Guar Gum	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	0.39 mg/ml	58.66 ± 1.52	18.89
2.	0.78 mg/ml	57.66 ± 1.52	20.28
3.	1.56 mg/ml	56.13 ± 0.57	22.39
4.	3.12 mg/ml	56.03 ± 0.57	22.53
5.	6.25 mg/ml	55 ± 1.73	23.95
6.	12.5 mg/ml	55.66 ± 0.57	23.04
7.	25 mg/ml	54.52 ± 1.52	24.62
8.	50 mg/ml	51.66 ± 0.57	28.57
9.	100 mg/ml	50.33 ± 0.57	30.41
10.	200 mg/ml	49.33 ± 2.08	31.79
11.	Control	72.33 ± 0.51	

Table 5: *Antifungal activity of guar gum against Alternaria solani.*

S. No.	Gum acacia	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	0.39 mg/ml	57.33 ± 0.57	20.73
2.	0.78 mg/ml	55.33 ± 1.52	23.50
3.	1.56 mg/ml	52.33 ± 0.57	27.65
4.	3.12 mg/ml	52.33 ± 0.57	27.65
5.	6.25 mg/ml	51.66 ± 0.57	28.57
6.	12.5 mg/ml	50.33 ± 0.57	30.41
7.	25 mg/ml	49.33 ± 0.57	31.79
8.	50 mg/ml	48.66 ± 0.57	32.72
9.	100 mg/ml	48.33 ± 1.15	33.18
10.	200 mg/ml	47.33 ± 1.52	34.56
11.	Control	72.33 ± 0.51	

Table 6: *Antifungal activity of gum acacia against Alternaria solani.*

S. No.	Cow dung	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1.	0.39 mg/ml	58.33 ± 0.57	19.35
2.	0.78 mg/ml	57.66 ± 0.57	20.28
3.	1.56 mg/ml	55.33 ± 0.57	23.50
4.	3.12 mg/ml	54.33 ± 0.57	24.88
5.	6.25 mg/ml	53.33 ± 0.577	26.26
6.	12.5 mg/ml	52.33 ± 0.57	27.65
7.	25 mg/ml	50 ± 1	30.87
8.	50 mg/ml	49.33 ± 0.57	31.79
9.	100 mg/ml	48.66 ± 0.57	32.72
10.	200 mg/ml	45.66 ± 0.57	36.87
11.	Control	72.33 ± 0.51	

Table 7: *Antifungal activity of cow dung against Alternaria solani.*

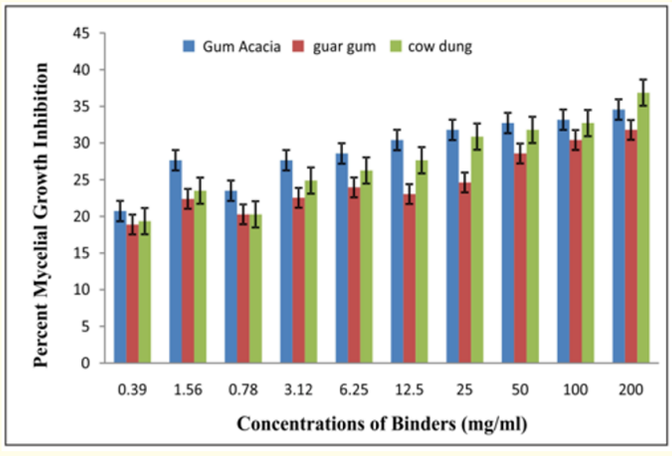


Figure 4: *Antifungal activity of binders against Alternaria solani.*

S. No.	Cow dung	Growth Diameter after 7days(mm) ± SD	% Mycelial Growth Inhibition
1.	10%	61.64 ± 0.36	14.77
2.	20%	60.13 ± 0.47	16.86
3.	30%	59.06 ± 0.57	18.34
4.	40%	57.21 ± 0.49	20.90
5.	50%	56.9 ± 0.48	21.36
6.	60%	55.33 ± 0.57	23.03
7.	70%	53.66 ± 0.57	25.81
8.	80%	51.33 ± 0.57	29.03
9.	90%	45.66 ± 1.15	36.87
10.	100%	42.33 ± 1.14	41.47
11.	Control	72.33 ± 0.51	

Table 8: *Antifungal activity of cow dung against Alternaria solani.*

S. No	Standard fungicides and water control	Growth Diameter after 7 days (mm) ± SD	% Mycelial Growth Inhibition
1	Mancozeb	15.33 ± 0.57	78.80
2	Bavistin	34 ± 1.73	52.99
3	Water	72.33 ± 2.51	

Table 9: *Antifungal activity of standard fungicides with water control against A. solani.*

Discussions

The use of botanicals for plant protection and pest management is becoming popular as control method [18]. Only use of plant extract in controlling plant disease is very expensive and a lot of plant parts are required for obtaining it. Use of elicitor and binders in combination with plant extracts is gaining importance because it is very cheap and has long term positive effect.

In the present study selection for best active binder and elicitor to get optimum antimicrobial efficiency has been studied. In this study four elicitors i.e. neem oil cake, mustard oil cake, coconut oil cake, and *Pongamia* oil cake etc. and three binders *i.e.* guar gum, gum *Acacia* and cow dung etc. were used to evaluate the antifungal activity against test pathogen. Many researchers have evaluated the antimicrobial activity of elicitors and binders against different pathogens as use of elicitor for control of plant diseases is ecofriendly and very effective [19]. Screened four oil cakes i.e. mahua cake extract, neem cake extract, castor and gingerly cake extracts against *Bipolaris oryzae,* the causal agent of brown spot disease in rice and amongst the all cake extracts neem cake extract showed the maximum inhibition of mycelial growth and spore germination followed by mahua cake extract, castor and ginger cake extract.

Oil cakes have also been reported for use in production of antibiotics and antimicrobials. Results suggested that oil cakes show concentration dependent antimicrobial activity. The same study pattern was also reported by some workers [20-24]. Antimicrobial potential of mustard oil cake, *Pongamia* oil cake has been studied by several workers [25,26]. In the present study significant inhibitory activity against *Alternaria solani* was observed with neem oil cake followed by *Pongamia* oil cake. The reason for this may be presence of specific secondary metabolites in neem which effect the growth and metabolism of the test fungus. Neem oil cake contains Azadirachtin, Azadirone, Gedunin, Meliacarpin, Nimbin, Salannin, Vilasinin which is known for the antifungal activity [27]. In the comparison, the secondary

metabolites found in other elicitors are not active or not enough to inhibit the growth of test fungus. This proves that growth inhibition by secondary metabolites is very specific and probably depends on the ligand binding with surface protein of the test fungus [28,29].

The next step in the process of exploring a suitable binder for enhancing the antimicrobial activity of plant extract into culture medium the poison food technique was used. Three binders namely guar gum, gum *Acacia* and cow dung etc were used to test the antifungal activity against test pathogen. All of these binders have antimicrobial activity against different pathogens but lower than oil cakes. Among the all binders, cow dung showed best inhibition against *Alternaria solani*. Cow dung possess antimicrobial properties against different pathogens. A lot of researchers have contributed in this field [30-32].

Some workers have reported significant antibacterial activity of saponin rich guar gum against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* [33]. Gum *Acacia* is obtained from *Acacia nilotica* Linn. All part of the plant i.e. gum, stem bark, leaves and fruits shows medicinal properties. Gaur gum and Gum *Acacia* also showed inhibitory activity but at very low concentration [34]. Various researchers evaluated the antifungal activity of guar gum, gum *Acacia* and cow dung against *Alternaria solani.*

Results indicate that best active elicitor i.e. neem oil cake and binder i.e. cow dung can be used to develop the plant extract based bioformulation for effective control of early blight of tomato in an eco-friendly manner.

Conclusion

On the basis of results obtained it can be concluded that use of oilcakes offers good alternative to traditional applications in the production of environment friendly bioformulation. Various binders are also eco-friendly option as their availability is very cheaper throughout the year.

Conflicts of Interest

There are no conflicts of interest. As this is my original research work.

Research Involving Human Participants and/or Animals

In this study no use of any kind of animals for the experimental purpose.

Authors Contribution Statement

Ms. Surbhi Mehta perceived the idea, carried out the research study, evaluated the results and drafted the manuscript. Prof. Kanika Sharma guided to Ms. Mehta in conducting this research study and also reviewed and approved the manuscript.

Funding Support

As the author do not get any kind of fellowship or scholarship, so there is no need to acknowledge any funding agency. The expenditures will be afforded by the corresponding author only if any.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Bibliography

1. El-Gamal NG., *et al*. "Induction of systemic resistance in potato plants against late and early blight diseases using chemical inducers under greenhouse and field conditions". *Research Journal of Agriculture and Biological Sciences* 3 (2007): 73-81.

2. Dhara P., *et al*. "Cameotra Biosurfactants in agriculture". *Applied Microbiology and Biotechnology* 97 (2013): 1005-1016.

3. Ebel J and Cosio EG. "Elicitors of plant defense responses". *International Review of Cytology* 148 (1994): 1-36.

4. Nurnberger T. "Signal perception in plant pathogen defense". *Cellular and Molecular Life Sciences* 55 (1999): 167-182.

5. Bektas Yand Eulgem T. "Synthetic plant defense elicitors". *Plant Physiology* 5 (2015): 804.

6. Santamaría ME and Ana Arnaiz A. "Arabidopsis response to the spider mite Tetranychus urticae depends on the regulation of reactive oxygen species homeostasis". *Scientific Reports* 8 (2018): 1-12.

7. Thomas Boller and Georg Felix. "A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors". *Annual Review of Plant Biology* 60 (2009): 379-406.

8. Aparna KP and Girija VK. "Efficacy of Plant Oils and Oil Cakes against Rhizoctonia solani Kuhn Causing Collar Rot and Web Blight of Cowpea under in vitro Conditions". *International Journal of Current Microbiology and Applied Sciences* 7.1 (2018): 2091-2098.

9. Rastogi RP and Mehrotra BN. "Compendium of Indian Medicinal Plants". New Delhi, NISC (2001): 4-7.

10. Angadi SV., *et al*. "Adaptation of alternative pulse and oilseed crops to the semiarid Canadian Prairie: Seed yield and water use efficiency". *Canadian Journal of Plant Science* 88 (2008): 425-438.

11. Tripathy and Das. "Guar gum: present status and applications". *The Journal of Pharmaceutical and Scientific Innovation* 2 (2013): 24-28.

12. Narayan DP and Purohit SS. "Agro's colour atlas of medicinal plants". *India Agrobios* 3 (2004).

13. Nadkarni KM. "The Indian Plants and Drugs". Shrishti Book Distributors, New Delhi (2005): 4-5.

14. Kamini K and Shuchi M. "Formulation and percentage evaluation of gum -Acacia as a binder W.S.R. to jwaraghani gutika (Herbo-Mineral Preparation)". *International Journal of Research in Medical Sciences* 5 (2016): 17-20.

15. Naskar Sethuraman SK and Ray PR. "Sprouting in plants by cow dung slurry. Validation of Indigenous Technical Knowledge in Agriculture Extension". *Indian Council of Agricultural Research* (2003): 197-201.

16. Basak AB and Lee MW. "*In vitro* inhibitory activity of cow urine and cow dung of Fusarium solani f. sp. Cucurbitae". *Mycobiology* 30 (2002): 51-54.

17. Groover RK and Moore JD. "Toxicometric studies of Fungicides against the brown root organisms Sclerotinia fructicola and S. laxa". *Phytopathology* 52 (1962): 876-880.

18. Ashraf Z., *et al*. "*In Vitro* Antibacterial and Antifungal Activity of Methanol, Chloroform and Aqueous Extracts of Origanum vulgare and Their Comparative Analysis". *International Journal of Organic Chemistry* 1 (2011): 257-261.

19. Harish S., *et al*. "Use of plant extracts and biocontrol agents for the management of brown spot disease in rice". *Bio Control* 53 (2007): 555-567.

20. Shukla PK and Haseeb A. "Effectiveness of some nematicides and oil cakes in the management of Pratylenchus thornei on Mentha citrata, M. piperita and M. spicata". *Bioresource Technology* 57 (1996): 307-310.

21. Khan TA and Saxena SK. "Integrated management of root knot nematode Meloidogyne javanica infecting tomato using organic materials and Paecilomyces lilacinus". *Bioresource Technology* 61 (1997): 247-250.

22. Omar JMA. "Effects of feeding different levels of sesame oil cake on performance and digestibility of Awassi lambs". *Small Ruminant Research* 46 (2002): 187-190.

23. Rao VK., *et al*. "Effect of feeling washed neem (Azadirarachta indica) seed kernel cake on the quality, lipid profile and fatty acid composition of goat meat". *Small Ruminant Research* 47 (2003): 213-219.

24. Jothi G., *et al*. "Management of root lesion nematode, Pratylenchus delattrei in crossandra using oil cakes". *Bioresource Technology* 93 (2004): 257-259.

25. Zrybko CL., *et al*. "Determination of glucosinolates in domestic and wild mustard by high-performance liquid chromatography and photodiode-array detection". *Journal of Chromatography A* 767 (1997): 43-52.

26. Mukherjee PS and Nandi B. "Preservation of seeds with some antifungal volatile compounds". *Journal of Mycopathological Research* 36 (1998): 25-29.

27. Hatti SS., *et al*. "Effect of Eisenia fetida vermiwash on the growth of plants". *Journal of Crop Science* 1 (2010): 6-10.

28. Hamini KN., *et al*. "Antifungal activity of clove (Syzygium aromaticum L.) essential oil against phytopathogenic fungi of tomato (Solanum lycopersicum L) in Algeria". *Journal of Experimental Biology and Agricultural Sciences* 2 (2014): 447-454.

29. Darwiche R., *et al*. "Plant pathogenesis-related proteins of the cacao fungal pathogen Moniliophthora perniciosa differ in their lipid-binding specificities". *Journal of Biological Chemistry* 292 (2017): 20558-20569.

30. Adegunloye DV., *et al*. "Microbial Analysis of Compost Using Cowdung as Booster". *Pakistan Journal of Nutrition* 6.5 (2007): 506-510.

31. Yongabi KA., *et al*. "Preliminary study on the effect of anaerobically digested cow dung slurry on the antimicrobial activity of three medicinal plants". *African Journal of Microbiology Research* 3 (2009): 168-174.

32. Joseph B and Sankarganesh P. "Antifungal Efficacy of Panchagavya". *International Journal of PharmTech Research* 3 (2011): 585-588.

33. Hassan SM., *et al*. "Haemolytic and antimicrobial activity of saponin rich extracts from guar meal". *Food Chemistry* 119 (2010): 600-605.

34. Hada D and Sharma K. "Comparative analysis of different elicitors and binders and development of herbal formulation containing Cassia fistula L. fruit pulp against Alternaria Solani". *The International Journal of Agricultural Science Research* 6 (2016): 371-380.

Volume 9 Issue 12 December 2021