www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 1312-1318

www.thepharmajournal.com
Received: 18-12-2022
Accepted: 20-01-2023

Ghodke SD
Agricultural Microbiology Section, Vasantdada Sugar Institute, Pune, Maharashtra, India

Shinde AS
Agricultural Microbiology Section, Vasantdada Sugar Institute, Pune, Maharashtra, India

Patil PM
Agricultural Microbiology Section, Vasantdada Sugar Institute, Pune, Maharashtra, India

Corresponding Author:
Ghodke SD
Agricultural Microbiology Section, Vasantdada Sugar Institute, Pune, Maharashtra, India

Efficacy of microbial bio weedicide for control of weeds in sugarcane

Ghodke SD, Shinde AS and Patil PM

Abstract
Microbial weedicide for the management of agricultural weeds is an eco-friendly approach. A worldwide programme has been growing up to control the invasive weed species for the better crop production and stable ecosystem. In India, yield losses due to weeds are more than those from pests and disease. Bioweedicide can be the best replacement for all the methods and is the safest method but not used in agriculture till date. Many fungi and bacteria produce several elements that are toxic to weeds in sugarcane. Bacteria isolated from diseased weeds were used as bioweedicide and consortium was prepared. Leaf detachment assay, mini plot and field trials studies were carried out to test the efficacy of bioweedicide. Effect of bioweedicide showed yellowing, scorching and blackening of leaves within 24 to 72 hours after foliar spray.

Keywords: Indian mustard, path coefficient analysis

Introduction
Weeds compete with crops for all the inputs and the total actual economic loss, due to weeds in 10 major crops of India, was estimated at US$ 11 billion (Gharde *et al.* 2018) [28]. Hence managing weeds is critical in attaining higher productivity of crops with improved resources use efficiency, to meet the food and nutritional demands of increasing Indian population as well as increasing income of the farmers (Rao and Chauhan 2015) [29]. Weed management involves integrated efforts to manage weeds in crops to selectively minimize the weed competition so as to enable crops to optimally use resources such as soil fertility, water and sunlight, for attaining the optimal harvestable crop yield. Biological control of weeds is the intentional use of living organisms (biotic agents) to reduce the vigor, reproductive capacity, density, or impact of weeds (Quimby and Birdsall, 1995) [30]. Therefore, the weed management practice adopted should ensure a weed free field condition for the first 3-4 months period. Poor growth of sugarcane resulting from infestation also affects quality. Certain fungal, bacterial, and viral pathogens can be mass produced and used as a biological weedicide to kill weeds in crops. Sugarcane is most susceptible to weed competition. Chemical herbicides show lots of side effects such as their long persistent period. It is necessary to develop eco-friendly weedicides. Some fungi and bacteria produce toxins against weeds. Toxins can be small molecules, peptides, or proteins that are capable of causing disease on contact with or absorption by body tissues interacting with biological macromolecules such as enzymes or cellular receptors. Endotoxins are large molecules consisting of a lipid and a polysaccharide composed of O-antigen, outer core and inner core joined by a covalent bond. They are found in the outer membrane of Gram negative bacteria. These toxins can be used as bioweedicide. These toxins are helpful in controlling weeds which are found in sugarcane field. Triazine exposure has been implicated in a likely relationship to increased risk of breast cancer, although a causal relationship remains unclear. The risk of Parkinson's disease has been shown to increase with occupational exposure to herbicides and pesticides. So as to minimize disadvantages we need to discover new strategies to control weeds from sugarcane. The attempt is to develop the eco-friendly & cost effective technology for controlling weeds in sugarcane.

Material and Methodology
Different diseased weeds were collected from sugarcane field of various locations. Pathogens were isolated and purified from diseased weed, using differential media by streak plate method (on Nutrient Agar Medium) for bacteria and spot inoculation (on Potato Dextrose Agar Medium) for fungi. Isolates were checked for their pathogenicity by using detached leaf

method on *Parthenium hysterophrous* leaves. Morphological study was done as per Bergy's manual. Culture broth was prepared by inoculating specific pathogen and Culture Filtrate (CF) of Fungi and Bacteria were done. The bacteria were cultured in 250 ml flask with 50 ml nutrient broth on a rotary shaker at 200 rpm for 24 h at 28 °C. The cells were harvested by centrifugation at 10,000 rpm for 10 min and supernatants were filtered through Whatman membrane (2.4 µM) (Katsumi Akutsu *et al*., 1993) [31] Mycelial disks of each a micro-organism grown on PDA was separately inoculated into 100 ml flasks containing potato dextrose broth and incubated at 25 to 29 °C for 15 days. The cultures were then filtered through Whatman filter paper. Then culture filtrates were used for germination test at different concentrations (Doustmorad Zafari *et al*., s2008). G.J. B.A.H.S., Vol. 1(2) 2012: 40-45 ISSN - 2319 – 5584 41. Bio-efficacy testing of Bioweedicide at laboratory scale was carried out by leaf detachment method. *Parthenium* leaves were detached and washed repeatedly by distilled water and 0.2% $HgCl_2$. Filter paper was placed on petriplate in sterile condition and *Parthenium* leaf was placed, application of bioweedicide was done in 3 replication (Sharma *et al.*, 2004) [32].

Mini plot trials (30x30 cm) on different weeds by foliar application @150 ml/plot. Plot were selected on Manjari farm marking (30x30 cm) was done. Foliar application of bioweedicide was done in 3 replication. Observations were recorded after every 24hrs for one week.

Foliar application of bioweedicide on *Cyperus rotundus* and *Cynodon dactylon* was carried out.

Field trials (3 m x 3 m) were conducted on different weeds by foliar application @ 3 lit/plot. Plots were selected for trial at Manjari farm and layout was prepared in randomized block design. Treatments were confirmed in 3 replications. Foliar application of bioweedicide was taken as per treatment details scheduled. Treatments were T1-Abs. Control (Weedi check)**,** T2-Control- weed free-check (Normal practices of weed control-hand weeding control), T3-Spraying of Bioweedicide 300 lit/ha**,** T4-Spraying of Bioweedicide 450 lit/ha**,** T5-Spraying of Bioweedicide 600 lit/ha**,** T6-Spraying of Bioweedicide 750 lit/ha**,** T7- Spraying of 2, 4 D- 1.250 kg/ha. T8-Spraying of Bioweedicide 900 lit/ha.

Bioweedicide trial on mixed weed in Sugarcane (Product Compatibility trial) at Manjari Farm was conducted in FRBD design with eight treatments in two replications. The gross plot size was 6.50 M x 5.48 M. (4 rows)-35.62m^2 and net plot size was 6 M x 2.74 M. (2 rows)-16.44m2). Two eye bud sets were planted with recommended spacing of 15 cm. The sugarcane variety used was VSI08005.

Foliar application was done according to following treatments: R1T8 – Abs. Control (Weedi check)**,** R1BwT13 – Pre-emergence bioweedicide application. R2V+BwT13 – Pre-emergence Oligochitosan + bioweedicide application. R1BwT14 – Post emergence bioweedicide application. R2 V + BwT14 –Post emergence Oligochitosan + bioweedicide application. R1BwT15 – Pre & Post- emergence bioweedicide application. R2 V+BwT15 –Pre & Post- emergence Oligochitosan + bioweedicide application. R2T8 – Spraying of Atrazine@ 2 kg ai/ha.

FYM was added @ 25 MT/ha to all treatments. Bioweedicide was applied as foliar application

Results & Discussion

Effect of bioweedicide on *Parthenium hysterophrous* by leaf detachment assay by "Koch's postulates": Blackening of leaf was observed after 24hrs.

Fig 1: Control

Fig 2: Application of BW (R1)

Fig 3: Application of BW (R2)

Fig 4: Control

Fig 5: Application of BW

Fig 6: Control

Fig 7: Application of Bw

Fig 8: Control

Fig 9: Application of Bw (R1)

Fig 10: Application of Bw (R2)

Fig 11: Application of Bw (R3)

Field trial - Effect of bioweedicide on *Cyperus rotundus* and *Cynodon dactylon* after 48hrs: Yellowing of leaves and scorching was observed after 48hrs and growth was stunted. Revival of weeds was not observed but new weed germinated place after two weeks.

Fig 12: Before application of BW

Fig 13: After application of BW

Field trial of bioweedicide for control of different weeds in wheat crop at manjari farm.

Table 1: Weed count of *Portulaca oleracea*, *Parthenium hysterophrous* and *Cyperus rotundus*

Treatment No.	*Portulaca oleraceae* (Days)						*Parthenium hysterophrous* (Days)						*Cyperus rotundus* (Days)					
	Pre	3	7	9	11	25	Pre	3	7	9	11	25	Pre	3	7	9	11	25
Absolute control	14	20	23	23	29	27	40	41	48	48	54	54	10	10	14	14	16	4
Hand weeding	13	1	3	3	6	7	21	8	11	11	17	31	14	10	12	12	17	6
Bw 300 lit/ha	37	19	16	16	18	12	69	18	39	39	42	54	16	12	10	10	18	5
Bw 450 lit/ha	11	9	9	9	12	16	91	37	44	44	54	57	22	12	30	30	47	10
Bw 600 lit/ha	40	22	22	22	17	7	62	19	16	16	27	35	24	21	21	21	9	7
Bw 750 lit/ha	26	14	16	16	11	14	94	17	27	27	50	66	43	22	25	25	32	14
2, 4 D- 1.250kg/ha	26	14	7	7	6	3	76	20	24	24	29	1	6	6	5	5	3	3
Bw 900 lit/ha	35	19	29	29	21	17	99	20	25	25	38	55	5	2	8	8	11	6

Table 2: Weed count of Amaranthus, Launaea procumbens, Broad leaves weed and Binding Weed.

Treatment No.	*Amaranthus* (Days)						*Launaea procumbens* (Days)						*Broad leaves* (Days)						*Binding spp.* (Days)					
	Pre	3	7	9	11	25	Pre	3	7	9	11	25	Pre	3	7	9	11	25	Pre	3	7	9	11	25
Absolute control	7	7	9	9	10	7	-	1	-	-	-	4	-	-	-	-	-	18	1	1	-	-	-	1
Hand weeding	3	2	8	8	11	5	1	1	-	-	-	1	2	-	-	-	-	23	1	1	-	-	-	2
Bw 300 lit/ha	6	3	1	1	3	11	-	-	-	-	-	3	3	-	-	-	-	-	-	-	-	-	-	1
Bw 450 lit/ha	6	5	3	3	4	13	-	-	-	-	-	3	-	-	-	-	-	21	-	-	-	-	-	-
Bw 600 lit/ha	1	1	1	1	4	11	-	-	-	-	-	1	-	-	-	-	-	19	-	-	-	-	-	-
Bw 750 lit/ha	-	2	2	2	5	19	3	-	-	-	-	6	-	-	-	-	-	14	4	-	-	-	-	-
2, 4 D- 1.250 kg/ha	9	3	-	-	-	3	1	-	-	-	-	-	2	-	-	-	-	6	2	-	-	-	-	-
Bw 900 lit/ha	6	1	2	2	4	4	-	-	-	-	-	4	2	-	-	-	-	13	1	-	-	-	-	1

Table 3: Weed count of *Shppi spp.* and, *Prikly Amaranth.*

Treatment No.	*Shippi spp.* (Days)						*Prikly Amaranth* (Days)					
	Pre count	3	7	9	11	25	Pre count	3	7	9	11	25
Absolute control	35	38	46	46	53	1	2	2	-	-	-	-
Hand weeding	42	9	15	15	20	-	-	-	-	-	-	-
Bw 300 lit/ha	70	64	34	34	35	-	-	-	-	-	-	-
Bw 450 lit/ha	67	60	34	34	29	-	1	-	-	-	-	-
Bw 600 lit/ha	83	70	59	59	19	-	-	-	-	-	-	-
Bw 750 lit/ha	52	44	33	33	22	-	2	-	-	-	-	-
2, 4 D- 1.250kg/ha	67	46	16	16	13	-	-	-	-	-	-	-
Bw 900 lit/ha	71	62	52	52	24	2	1	-	-	-	-	-

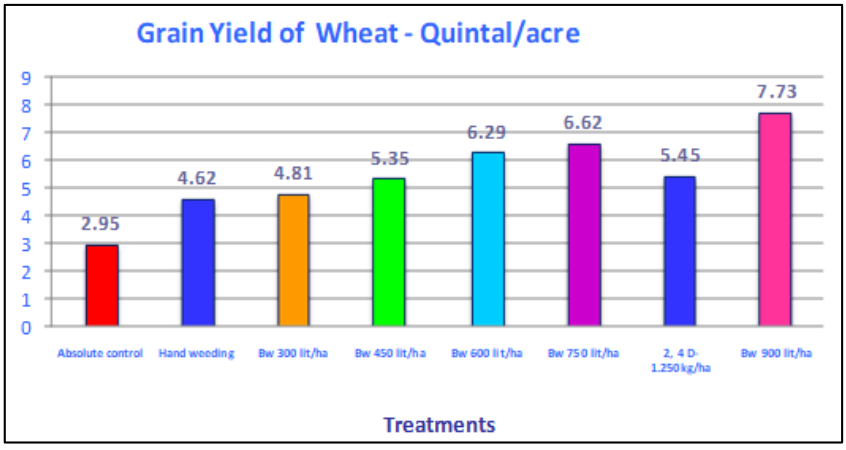

Fig 14: Grain Yield of Wheat

Field trial of bioweedicide for control of different weeds in wheat crop showed stunted growth of *Portulaca oleraceae, Parthenium hysterophrous, Cyperus rotundus, Amaranthus* and *Shippi*. Some weeds showed scorching and yellowing. No revival was observed but after 9 days new weed were germinated. In case of *Binding weed* and *Shippi* weed, scorching was observed and growth was stunted. *Cynodon dactylon* weed showed 100% death and no revival of weed or no any new weed germination was observed. Here, highest grain yield is observed in 'T8' i.e spraying of bioweedicide 8361lit/ha and lowest grain yield is observed in 'T1' i.e. control. Here, highest grain yield is observed where spraying of bioweedicide @ 900lit/ha and lowest grain yield is observed in control.

Compatibility trial for control of different weeds in sugarcane field at manjari farm

Table 4: Average Weed count of pre-emergence application of Bioweedicide alone & in combination with Oligochitosan

Bioweedicide application	Pre	Day 3	Day 5	Day 7	Day 9	Day 30	Bioweedicide + Oligochitosan	Pre	Day 3	Day 5	Day 7	Day 9	Day 30
Ipomea spp	20	12	6	8	9	36	*Ipomea spp*	89	38	14	41	55	106
Purslane	94	3	30	11	23	35	*Purslane*	109	25	66	67	62	172
Cyperus rotundus	2	1	1	2	3	-	*Cyperus rotundus*	8	8	17	17	24	-
Day flower	1	-	1	-	1	18	*Day flower*	1	-	-	-	-	-
Parthenium	20	7	19	7	14	35	*Parthenium*	18	-	1	2	3	54
Shippi	1	5	25	47	76	133	*Shippi*	-	4	14	50	71	97
Amaranthus	-	-	-	2	2	71	*Amaranthus*	-	-	-	1	3	71
Amaranthus spinosus	-	-	-	-	-	18							
Euphorbia	-	-	-	1	1	-							

Table 5: Average Weed count of post-emergence application of Bioweedicide alone & in combination with Oligochitosan

Bioweedicide alone	Pre count	24 hrs	Day 3	Day 5	Day 7	Day 9	Day 30	Bioweedicide + Oligochitosan	Pre count	24 hrs	Day 3	Day 5	Day 7	Day 9	Day 30
Ipomea spp	22	12	13	25	25	18	18	*Ipomea* spp	34	29	29	49	49	71	71
Purslane	160	36	11	34	34	44	65	*Purslane*	158	87	50	127	127	177	177
Cyperus spp	3	1	7	5	5	12	-	*Cyperus spp*	15	11	10	15	15	-	-
Parthenium	68	1	4	21	21	70	71	*Day flower*	1	1	1	1	1	-	-
Shippi	121	59	137	145	145	388	391	*Parthenium*	76	64	92	124	124	213	213
Amaranthus	65	-	6	47	47	61	61	*Shippi*	172	181	235	258	258	445	445
Amaranthus spinosus	-	-	1	1	1	18	18	*Amaranthus*	9	23	8	17	17	160	160
Euphorbia	1	-	-	-	-	-	-	*Argemona mexicana*	1	1	1	1	1	-	-

The small weeds were affected by bioweedicide alone and weed rate was decreased. Scorching, stunted growth, yellowing of leaves of weeds was observed. Few weed had stunted growth. After one week, new weed germinated.

Scorching and yellowing of leaves was observed by application of Bioweedicide in combination with Oligochitosan but there was also revival of weeds and also showed very high new weed germination after only 3 days of application. Even though weeds were affected by bioweedicide within 24 hrs, some of them showed revival (Table no. 4 & 5). Sugarcane shoot affected by bioweedicide were slight and very few were moderate and recovery was possible (Table no. 6). Also there was no complete destruction of sugarcane plant due to application of bioweedicide.

Table 6: Number of affected sugarcane shoots by application of bioweedicide

Sr. No.	Number of affected sugarcane shoots			
	R1		R2	
	Bioweedicide +Oligochitosan	Bioweedicide	Bioweedicide +Oligochitosan	Bioweedicide
1	2	2	3	2
2	3	1	1	1
3	1	2	1	1
4	4	1	1	1
5	1	1	2	2
6	3	1	-	2
7	1	1	-	1
8	2	2	-	-
9	1	-	-	-
10	1	-	-	-
11	3	-	-	-
Total no. of germinated sugarcane	18	19	14	15

Ratings: 1: Slight stunting injury or discoloration, 2: Some stand loss, stunting/ discoloration, 3: Injury more pronounced but not persistent, 4: Moderate injury, recovery possible, 5: Injury more persistent, recovery doubtful, 6: More severe injury, no recovery possible, 7: Severe injury, stand loss, 8: Almost destroyed few part surviving, 9: Very few part alive, 10: Complete destruction

Conclusion

The classical bio-control approach using plant pathogen has been successful over weed control.

It is concluded from the experiment that bacterial isolated from infected weed have the potential of preventing or inhibiting the germination of weed also infecting weed by stunting their growth, scorching and forming lesions.

The mini plot trials and field trials confirmed control of weeds by bioweedicide.

Acknowledgements
This work was supported by the Advisor and Director General of Vasantdada Sugar Institute, Pune. We are grateful of them.

References

1. Abu-Dieyeh MH, Watson AK. Efficacy of Sclerotinia minor for dandelion control: Effect of dandelion accession, age and grass competition. Weed Res. 2007a;47:63-72. 10.1111/j.1365-3180.2007.00542.x [CrossRef] [Google Scholar]
2. Abu-Dieyeh MH, Watson AK. Grass overseeding and a fungus combine to control *Taraxacum officinale*. J. Appl. Ecol. 2007b;44:115-124. 10.1111/j.1365-2664.2006.01247.x [CrossRef] [Google Scholar]
3. Andolfi A, Cimmino A, Villegas-Fernandez AM, Tuzi A, Santini A, Melck D, *et al*. Lentisone, a new phytotoxic anthraquinone produced by Ascochyta lentis, the causal agent of Ascochyta Blight in Lens culinaris. J. Agric. Food Chem. 2013;61:7301–7308. 10.1021/jf4026663 [Pub Med] [CrossRef] [Google Scholar]
4. Auld BA, Hetherington SD, Smith HE. Advances in bioherbicide formulation. Weed Biol. Manag. 2003;3:61-67. 10.1046/j.1445-6664.2003.00086.x [CrossRef] [Google Scholar]
5. Auld BA, McRae CF, Say MM. Possible control of *Xanthium spinosum* by a fungus. Agric. Ecosyst. Environ. 1988;21:219-223. 10.1016/0167-8809(88)90089-8 [CrossRef] [Google Scholar]
6. Auld BA, Morin L. Constraints in the development of bioherbicide. Weed Technol. 1995;9:638-652.
7. Auld BA, Say MM, Ridings HI, Andrews J. Field applications of *Colletotrichum orbiculare* to control *Xanthium spinosum*. Agric. Ecosyst. Environ. 1990;32:315-323. 10.1016/0167-8809(90)90168-d [CrossRef]
8. Bailey KL, Falk S, Derby JA, Melzer M, Boland GJ. The effect of fertilizers on the efficacy of the bioherbicide, *Phoma macrostoma*, to control dandelions in turfgrass. Biol. Control. 2013;65:147-151. 10.1016/j.biocontrol.2013.01.003 [CrossRef] [Google Scholar]
9. Mycotoxins: Producing Fungi and Mechanisms of Phytotoxicity Ahmed A. Ismaiel and Jutta Papenbrock
10. Bailey KL, Pitt WM, Falk S, Derby J. The effects of *Phoma macrostoma* on non-target plant and target weed species. Biol. Control. 2011;58:379-386. 10.1016/j.biocontrol.2011.06.001 [CrossRef] [Google Scholar]
11. Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI. Germination-Arrest Factor (GAF): biological properties of a novel, naturally-occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol. Control. 2008;46:380-390. 10.1016/j.biocontrol.2008.04.016 [CrossRef] [Google Scholar]
12. Cecilie F, Rudiger P, Peter S, Vector H, Per Einar G. Toxin-producing ability among *Bacillus* spp. outside the bacillus cerus group. Appl. Environ. Microbiology
13. Chandrakala A, Chandrashekar SC, Jyothi G, Ravikumar BM. Department of Plant Pathology, University of Agricultural Sciences, GKVK, Karnataka, Bangalore-560 065, India.
14. Hoagland RE. Microbes and microbial products as herbicide- An overview. Pp 2-52 In R.E. Hoagland [ed.], ACS Symposium Series 439; Microbes and Microbial Products as Herbicides. American Chemical Society, Washington, DC; c1990.
15. Jai Gosh S, Sagar Barale S. Department of Microbiology, Shivaji University, Kolhapur, Maharashtra (M.S), India.
16. Li YQ, Sun ZL, Zhuang XF, Xu L, Chen SF, Li MZ. Research progress on microbial herbicides. Crop Protect. 2003;22:247-252. 10.1016/s0261-2194(02)00189-8 [CrossRef]
17. Manpreet Kaur, Neeraj Kumar Aggarwal, Vikas Kumar, Romika Dhiman. Kurukshetra University, Kurukshetra, Haryana 136119, India.
18. Stephen L, Mark SS, Kit J. Expression and secretion of the cloned pseudomonas aeruginosa exotoxin A by *Escherichia coli*. J Bacteriol. 1988;70(2):714-719.
19. Tiwari JP, Bisen GR. Growth analysis of *Parthenium hysterophrous* L. Indian Journal of Weed Science. 1982;14:49-53
20. https://jaipurbiofertilizers.wordpress.com/2018/01/02/the-harmful-effects-of-weedicides-and-their negation/#:~:text=Respiratory%20Damage,indications%20or%20perpetual%20basic%20harm.
21. Vidhasekeran P, Rangasamy P, Som Prakash M, bioherbicide development-an overview Crop Prot. 22:253-260
22. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6684332/
23. https://link.springer.com/article/10.1007/BF01922457
24. https://www.jstor.org
25. Chutia M, Mahanta JJ, Bhattacharyya N, Bhuyan M, Boruah P, Sarma TC. Microbial Herbicides for Weed Management: Prospects, Progress and Constraints. Plant Pathology Journal. 2007;6:210-218.
26. Xu Z, Shi M, Tian Y, Zhao P, Niu Y, Liao M. Dirhamnolipid Produced by the Pathogenic Fungus *Colletotrichum gloeosporioides* BWH-1 and Its Herbicidal Activity. Molecules. 2019;24(16):2969. doi: 10.3390/molecules24162969. PMID: 31426281; PMCID: PMC6720573.
27. https://www.researchgate.net/publication/326187541_The_Role_of_Bioherbicides_in_Weed_Management
28. Gharde Y, Singh PK, Dubey RP, Gupta PK. Assessment of yield and economic losses in agriculture due to weeds in India. Crop Protection. 2018 May 1;107:12-18.
29. Rao PS, Midde MN, Miller DD, Chauhan S, Kumar A, Kumar S. Diallyl sulfide: potential use in novel therapeutic interventions in alcohol, drugs, and disease mediated cellular toxicity by targeting cytochrome P450 2E1. Current drug metabolism. 2015 Jul 1;16(6):486-503.
30. Quimby PC, Birdsall JL. Fungal agents for biological control of weeds: Classical and augmentative approaches. Novel approaches to integrated pest management; c1995. p. 293-308.
31. Akutsu K, Hirata A, Yamamoto M, Hirayae K, Okuyama S, Hibi T. Growth inhibition of Botrytis spp. by Serratia marcescens B2 isolated from tomato phylloplane.

Japanese Journal of Phytopathology. 1993 Feb 25;59(1):18-25.
32. Sharma S, Sultana S. Effect of Hibiscus rosa sinensis extract on hyperproliferation and oxidative damage caused by benzoyl peroxide and ultraviolet radiations in mouse skin. Basic & clinical pharmacology & toxicology. 2004 Nov;95(5):220-225.