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In vitro evaluation of botanicals against ralstonia solanacearum causing bacterial wilt of potato

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Abstract
Bacterial wilt or brown rot of potato, caused by Ralstonia solanacearum, is a limiting factor of potato production worldwide. The management of the disease is difficult, and the chemicals are least effective and hazardous. Hence, it is needs to develop alternative methods to control this deadly disease, which is effective and eco-friendly. Aqueous and ethanolic extracts of eleven plants were evaluated for the antibacterial activity against Ralstonia solanacearum in in-vitro by well diffusion method. Out of the eleven crude aqueous extracts tested, maximum inhibition zone was observed in bulb extract of Allium sativum (30.21mm) followed by Psidium guajava (25.11mm), Curcuma longa (17.77mm), Tagetes erecta (16.21mm), Carica papaya (14.55mm) and Azadirachta indica (13.77mm). Whereas on evaluation of eleven crude ethanolic extracts, maximum inhibition zone was observed in bulb extract of Allium sativum (29.77mm) followed by Psidium guajava (22.88mm), Tagetes erecta (19.77mm), Carica papaya (15.11mm), Azadirachta indica (14.88mm), Ocimum gratissimum (14.10) and Curcuma longa (11.33mm). After screening, four aqueous extracts of botanicals (Allium sativum, Psidium guajava, Curcuma longa and Tagetes erecta) and four ethanol extracts of botanicals (Allium sativum, Psidium guajava, Tagetes erecta and Carica papaya) which showed inhibition zone ≥15mm were evaluated against R. solanacearum at1%, 5%, 10% and 15% concentration. Among them, garlic showed superiority over all the plants extracts at all the levels of concentration.

Keywords: botanicals, ralstonia, solanacearum, potato

Introduction
Potato (*Solanum tuberosum* L.) is one of the four major and important food crops after wheat, maize and rice around the world (Hawkes, 1992) [10]. It is a rich source of nutrients. Bacterial wilt or brown rot, caused by Ralstonia solanacearum, limit potato production throughout the world. It causes huge crop losses in tropical, subtropical, and warm temperate regions (Hayward, 1994; Elphinstone, 2005) [9, 3]. Verma and Shekhawat (1990) [17] reported nearly 37% of potato loss due to brown rot disease in potato in Mukteshwar region of Uttarakhand, 13.8-55% in Kumaun hills and 75% in Karnataka (Gadewar *et al*., 1991) [4].
The management of bacterial wilt disease caused by R. solanacearum is difficult due to its broad host range with the ability to survive in the soil and roots of non-host plants including several weeds. The available conventional bactericides and fumigants such as methyl bromide did not provide effective control of this disease on soil treatment (Chellemi *et al*., 1997; Enfinger *et al*., 1979) [1, 2]. By the use of plant metabolites bacterial wilt diseases of potato can be managed which is eco-friendly, non-hazardous and bio-degradable. India is a land of biodiversity in terms of plant species. Various plants have been mentioned in Ayurveda, an ancient Indian Sanskrit literature, for their therapeutic advantages (Kaushik, 1988) [11]. There are many reports by the different researcher that plant metabolites or plant- based pesticides can be used as a good alternative to manage the plant diseases as they have a minimal environmental impact and also non-hazardous in contrast to chemicals (Harborne,1998; Varma and Dubey, 1999; Gottlieb *et al*., 2002) [8, 16, 6]. The natural plant products derived from plant species have the capacity to control diseases caused by viruses, bacteria and fungal pathogens (Guleria and Tiku, 2009) [7]. Therefore, under this scenario, botanical extracts seem to be an ideal candidates to be exploited in the management of bacterial wilt of potato because of the safety, renewable nature, cost-effective and high target specificity. The objective of this study was to determine the efficacy of aqueous and ethanolic extract of various plants for controlling R. solanacearum causing bacterial wilt of potato under *in vitro* condition.

Materials and Methods
Isolation and purification of R. solanacearum strains from wilted potato plants Potato plants showing typical symptoms viz., wilting, stunting and yellowing of plants infected by bacterial

wilt were collected and diagnosis of the disease was done by ooze test. The pathogen was cultured on TZC (tetrazolium chloride) agar medium and well isolated typical colonies of an isolate of R. solanacearum were picked and streaked separately on TZC medium. The pure cultures of the isolates were preserved on CPG slants and kept at 40C for future use.

Sample Collection: Eleven local plants were used in the study. The parts of the plants that were used in this study are the most popular and most readily available. They are shown in Table 1.

Method of Extraction: Aqueous and ethanolic extraction was followed to extract the antimicrobial components contained in eleven different plant species to screen for their antimicrobial property against potato bacterial wilt pathogen.

Protocol for aqueous extract: The crude aqueous extracts of garlic, guava, marigold, turmeric, lemongrass, ginger, onion, neem, tulsi, papaya and datura were prepared according to the method described by Raghu *et al*. 2013 [14].

Protocol for ethanol extract: The crude ethanolic extracts of garlic, guava, marigold, turmeric, lemongrass, ginger, onion, neem, tulsi, papaya and datura were prepared according to the method described by Kumar *et al*. 2017 [12].

***In vitro* evaluation of botanicals extracts:** Both water and ethanolic plant extracts were tested for their antibacterial property by agar well diffusion method (Gopalakrishnan *et al*., 2014) [5]. The commercial antibiotic, Streptomycin 500ppm was used as standards for comparison. The well which was loaded with sterile distilled water was used as control. The experiment was performed in triplicate under aseptic conditions. The Petri plates were then placed in the lowermost shelf of a refrigerator (5 ^{0}C) for half an hour, thus allowing plant extracts and test chemicals to diffuse into the medium. The plates were then shifted to the incubator at 28 ±10C. The inhibition zone was measured in mm with the help of a scale after 24 h of incubation.

Evaluation of screened aqueous and ethanolic extracts of botanicals against R. solanacearum at different concentration

Further four aqueous plant extracts (garlic, guava, turmeric and marigold) and four ethanolic extracts (garlic, guava, marigold and papaya) producing an inhibition zone ≥15mm in diameter were made to different concentrations viz.,1 percent, 5 percent, 10 percent, 15 percent, by adding sterilized distilled water proportionately and screened to determine minimum inhibitory concentration.

Results and Discussion

Screening of crude aqueous extract of different botanicals against R. solanacearum. Aqueous extracts of different botanicals were selected to evaluate their antibacterial property against Ralstonia solanacearum. Data presented in table 2 showed that out of 11 botanicals extract, 6 botanicals extract (garlic, guava, marigold, turmeric, neem and papaya) exhibited antibacterial property whereas 5 botanicals extract (datura, onion, ginger, tulsi and lemongrass) failed to inhibit the growth of Ralstonia solanacearum *in vitro* (Plate-1). Out of the six botanicals extract, garlic bulb extract was highly inhibitory to the growth of the pathogen showing the highest mean inhibition zone of 30.21mm, followed by guava leaf extract with an inhibition zone of 25.11mm. The next best botanicals extract were turmeric, marigold, papaya and neem extracts with inhibition zones of 17.77mm, 16.21mm, 14.55 mm and 13.77mm, respectively (Fig.-1).

Several workers reported the inhibitory effects of botanicals against R. solanacearum *in vitro*. Sinha, K. (2016) [15] demonstrated *in vitro* evaluation of botanicals against R. solanacearum in which he had taken rhizome, bulb and leaf aqueous extracts. Among them, the aqueous extract of garlic (Allium sativum) at 100% concentration found to be very effective and showed an inhibition zone of about 21mm & 24mm in diameter at 24 & 48 hours after incubation.

Evaluation of aqueous extract of different botanicals at different concentration against R. solanacearum under *in vitro* conditions

Data presented in table 3 showed that out of the four botanicals extract tested, garlic bulb extract recorded maximum inhibition zone (9.06 mm) at 1% concentration whereas other botanicals extract failed to inhibit the growth of R. solanacearum. At 5% concentration, garlic extract exhibited the highest inhibition zone (11mm) followed by guava (8.38 mm) and turmeric (8.10mm) respectively; whereas marigold extract failed to inhibit the growth of R. solanacearum. At 10% concentration, garlic extract exhibited the highest inhibition zone (12mm) followed by marigold (10.10mm), guava (8.99mm), and turmeric (8.36) extracts respectively. At 15% concentration garlic extract exhibited highest inhibition zone (13.11) followed by extracts of marigold (11.33mm), guava (11.32mm), and turmeric (8.77mm) (Plate-2 and Fig.-2). The statistical analyses also showed that garlic showed superiority over all the botanicals extract at all the levels of concentration followed by marigold and guava which is statistically at par at 10 and 15% concentration. Gopalakrishnan *et al*., (2014) [5] demonstrated *in vitro* evaluation of botanicals against R. solanacearum in which they had taken 23 botanicals. Among them, garlic extract showed a higher zone of inhibition of about 49mm diameter at 10% concentration.

Screening of crude ethanol extracts of botanicals against R. solanacearum. Ethanol extracts of different botanicals were evaluated for their antibacterial property against Ralstonia solanacearum. Extracts were taken at 100% concentration. Observations were taken in the form of inhibition zone (mm) after 24 h of incubation. The results revealed that out of 11 botanicals extract tested, 7 botanicals extract (garlic, guava, marigold, turmeric, neem, papaya and tulsi) exhibited significant antibacterial property whereas 4 botanicals extract (datura, onion, ginger and lemongrass) failed to inhibit the growth of R. solanacearum *in vitro*. The result presented in table- 4 and plate-3.

Out of the seven botanicals extract garlic bulb extract exhibited maximum zone of inhibition (29.77mm) followed by guava leaf extract (22.88mm), marigold (19.77mm), papaya (15.11mm), neem (14.88mm), tulsi (14.10mm) and turmeric extracts (11.33mm) (Fig.-3). Similar findings were made by Owoseni and Sangoyomi (2014), who reported that A. Sativum ethanolic extracts had the widest zone of inhibition (12.5mm) out of 10 ethanolic extracts at 100% concentration.

Evaluation of ethanolic extract of botanicals at different concentration against R. solanacearum

Out of the four botanicals extract tested only garlic bulb exhibited antibacterial activity at 1% concentration and the inhibition zone produced by garlic extracts was 8.27 mm against R. solanacearum (Fig.-4). At 5 and 10% concentration garlic showed maximum inhibition zone (9.05 mm and 11.72 mm) and guava showed (8.05mm and 8.76mm) inhibition

zone respectively, whereas marigold and papaya extract failed to inhibit the growth of R. solanacearum respectively. At 15% concentration marigold and papaya also showed antibacterial activity against R. solanacearum but the maximum inhibition zone was recorded in garlic (13mm) followed by guava (10.94), marigold (8.66mm) and papaya (7.88mm). Result presented in table 5 and fig. 4. The statistical analysis also showed that garlic showed superiority over all the plant extracts at all levels of concentration followed by guava. However, marigold and papaya statistically at par at 15% concentration.

Conclusion

Eleven aqueous and ethanol extracts of botanicals were studied *in vitro* against R. solanacearum. Among aqueous extract, garlic extract (Allium sativum) at 100% concentration found to be very effective and showed the highest inhibition zone of about 30.21mm. After screening, four aqueous extracts of botanicals (garlic, guava, marigold and turmeric) which showed inhibition zone ≥15mm was evaluated against R. solanacearum at1%, 5%, 10% and 15% concentration. Among them, garlic showed superiority over all the botanicals extract at all the levels of concentration. In ethanol extract, garlic extract (Allium sativum) at 100% concentration produced maximum inhibition zone 29.77mm. After screening, four ethanol extracts of botanicals (garlic, guava, marigold and papaya) which showed inhibition zone ≥15mm was evaluated against R. solanacearum at 1%, 5%, 10% and 15% concentration. Among them, garlic showed superiority over all the botanicals extract at all the levels of concentration.

Table 1: List of botanicals used for the preparation of extracts

S. No.	Local name	Scientific name	Family	Plant parts used
1	Ginger	Zingiber officinale	Zingiberaceae	Rhizome
2	Neem	Azadirachta indica	Meliaceae	Leaf
3	Tulsi	Ocimum gratissimum	Lamiaceae	Leaf
4	Garlic	Allium sativum	Amaryllidaceae	Bulb
5	Onion	Allium cepa	Amaryllidaceae	Bulb
6	Guava	Psidium guajava	Myrtaceae	Leaf
7	Papaya	Carica papaya	Caricaceae	Leaf
8	Turmeric	Curcuma longa	Zingiberaceae	Rhizome
9	Lemon grass	Cymbopogon citratus	Poaceae	Leaf
10	Marigold	Tagetes erecta	Asteraceae	Leaf
11	Datura	Datura stramonium	Solanaceae	Leaf

Table 2: Effect of crude aqueous extract of different botanicals on R. solanacearum under *in vitro* condition

S. No.	Botanicals	Inhibition zone (mm) against R. solanacearum
1.	Neem	13.77
2	Tulsi	0
3.	Garlic	30.21
4.	Onion	0
5.	Guava	25.11
6.	Papaya	14.55
7.	Turmeric	17.77
8.	Lemon Grass	0
9.	Datura	0
10.	Marigold	16.21
11.	Ginger	0
12.	Streptomycin(500ppm)	11.72
13.	Control	0
SEm±		0.66
CD at 5%		1.93

Table 3: Effect of aqueous extract of different botanicals at the different concentrations on R. solanacearum under *in vitro* conditions.

S. No.	Botanicals	Inhibition zone (mm) against R. solanacearum			
		1%	5%	10%	15%
1.	Guava	0	8.38	8.99	11.32
2.	Garlic	9.06	11	12	13.11
3.	Marigold	0	0	10.10	11.33
4.	Turmeric	0	8.10	8.36	8.77
5.	Streptomycin 500ppm	11.72			
6.	Control	0			
Factors		CD at 5%		SEm±	
Botanicals (A)		0.88		0.30	
Concentratin (B)		0.72		0.25	
Interaction (A×B)		1.76		0.61	

Table 4: Effect of crude ethanol extract of different botanicals on R. solanacearum under *in vitro* conditions

S. No.	Botanicals	Inhibition zone (mm) against R. solanacearum
1.	Neem	14.88
2	Tulsi	14.10
3.	Garlic	29.77
4.	Onion	0
5.	Guava	22.88

6.	Papaya	15.11
7.	Turmeric	11.33
8.	Lemon Grass	0
9.	Datura	0
10.	Marigold	19.77
11.	Ginger	0
12.	Streptomycin(500ppm)	11.72
13.	Control	0
SEm±		0.47
CD at 5%		1.38

Table 5: Effect of ethanol extract of different botanicals at the different concentrations on R. solanacearum under *in vitro* conditions.

S. No.	Botanicals	Inhibition zone (mm) against R. solanacearum			
		1%	5%	10%	15%
1.	Guava	0	8.05	8.76	10.94
2.	Garlic	8.27	9.05	11.72	13
3.	Marigold	0	0	0	8.66
4.	papaya	0	0	0	7.88
5.	Streptomycin 500ppm	11.72			
6.	Control	0			
Factors		CD at 5%		SEm±	
Botanicals (A)		0.87		0.30	
Concentration (B)		0.71		0.25	
Interaction (A×B)		1.75		0.61	

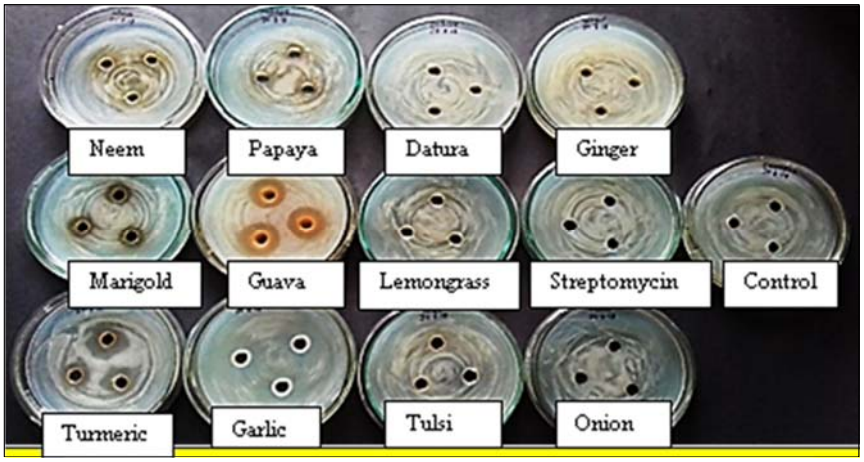
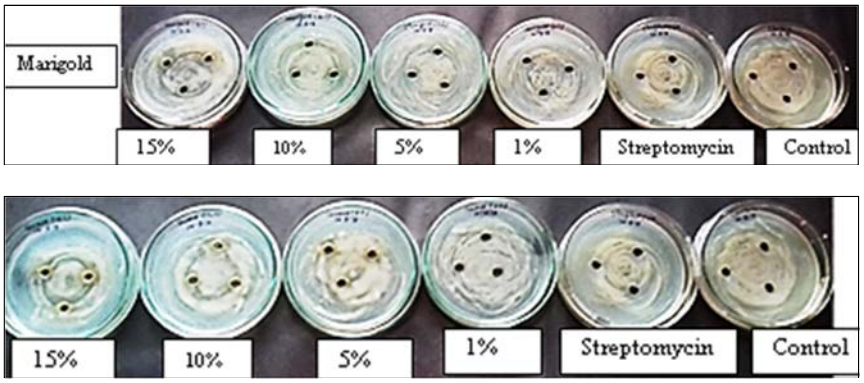


Plate 1: Inhibition zone produced by aqueous extracts of different botanicals against R. solanacearum at 100% concentrations



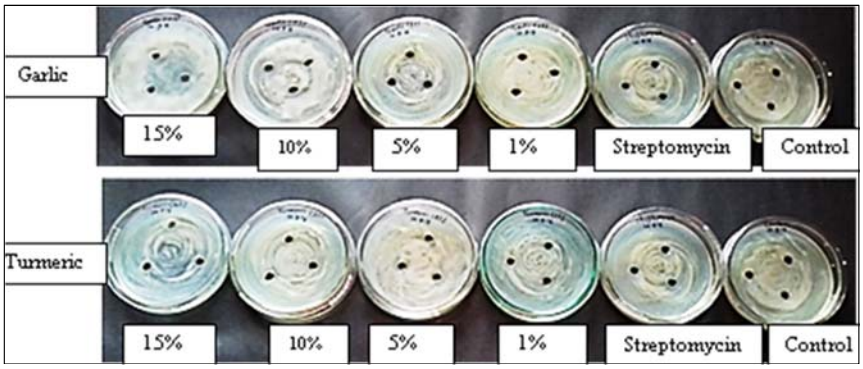


Plate 2: Inhibition zone produced by aqueous extracts of different botanicals against R. solanacearum at 1%, 5%, 10% and 15% concentrations

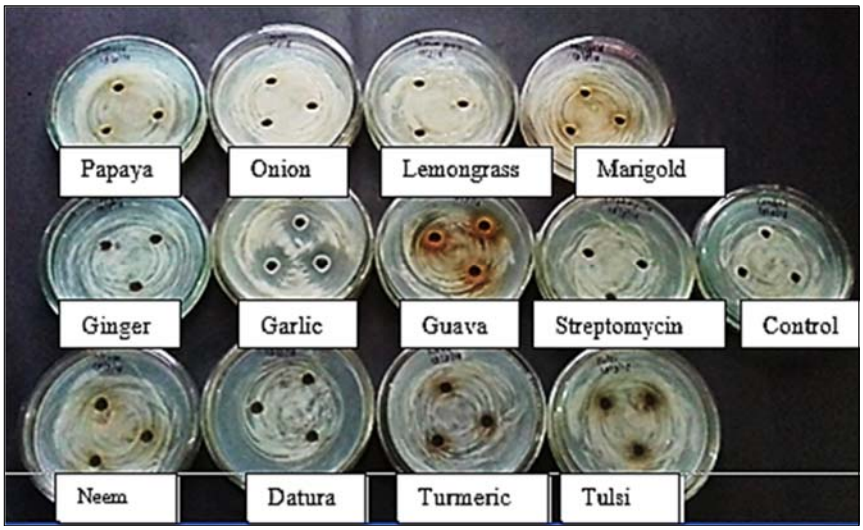


Plate 3: Inhibition zone produced by ethanol extracts of different botanicals against R. solanacearum at 100% concentrations

Plate 4: Inhibition zone produced by ethanol extracts of different botanicals against R. solanacearum at 1%, 5%, 10% and 15% concentrations

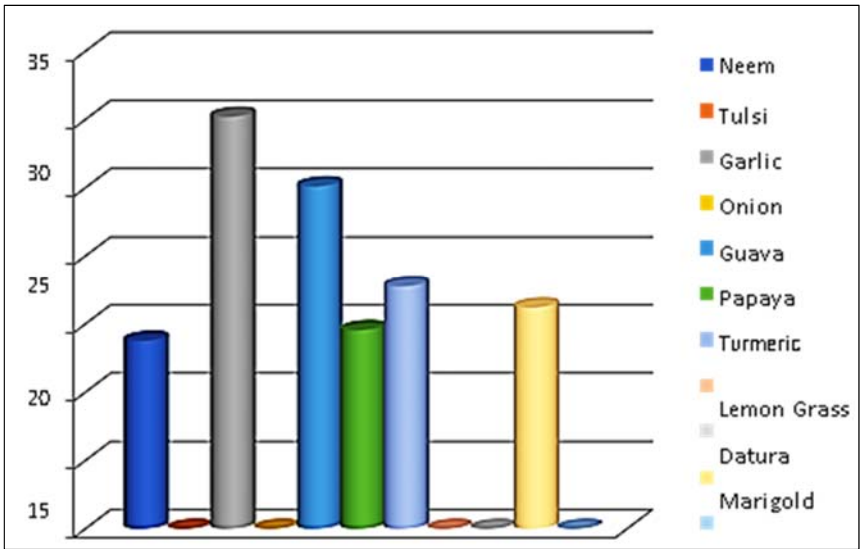


Fig 1: Inhibition zone produced by aqueous extracts of different botanicals against R. solanacearum at 100% concentrations under *in vitro* conditions

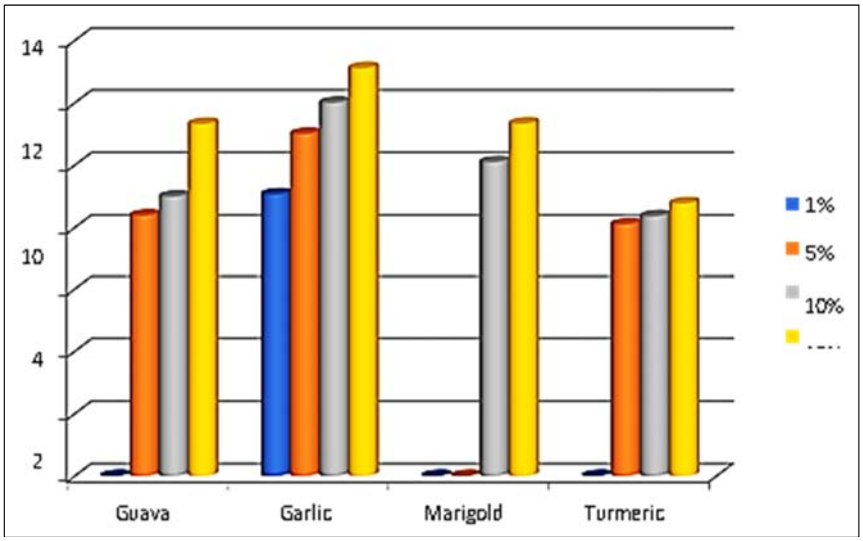


Fig 2: Inhibition zone produced by aqueous extracts of different botanicals against R. solanacearum at 1%, 5%, 10% and 15% concentrations under *in vitro* conditions

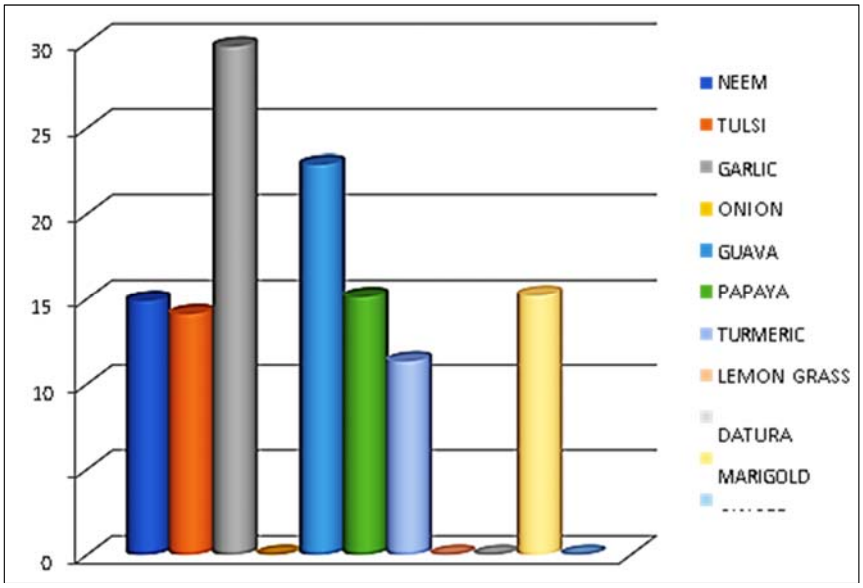


Fig 3: Inhibition zone produced by ethanol extracts of different botanicals against R. solanacearum at 100% concentrations under *in vitro* conditions

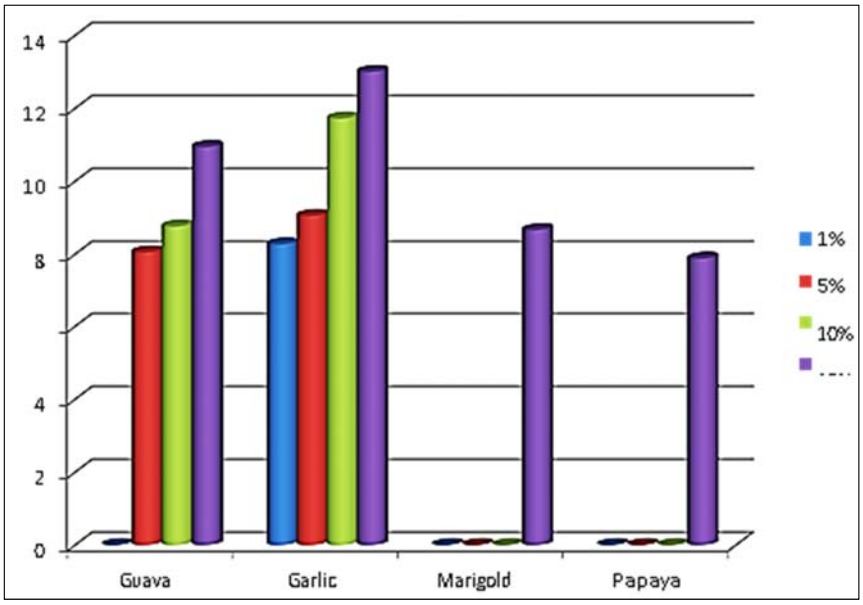


Fig 4: Inhibition zone produced by ethanol extracts of different botanicals against R. solanacearum at 1%, 5%, 10% and 15% concentrations under *in vitro* conditions

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