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Formulation of Allicin Based Disinfectant: Ant Repellent and *Brassica oleracea* Preservative Properties

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Abstract

The main objective of the work is to formulate an effective disinfectant. The main compounds of the disinfectant are extracted from natural sources and to study its effectiveness in vegetable preservation. Pathogens in the environment are responsible for the spoilage and contamination of food, which can be reduced by disinfectant. However synthetic disinfectant possess threat to human health and environment. Natural disinfectant is formulated by extracting allicin, a phenolic compound from garlic (*Allium sativum)*, mint extract from peppermint leaves (*Mentha piperita)*, saponin (surfactant) from coconut (*Cocos nucifera)* leaves. These compounds are safe to be used and effective against pathogens. The effectiveness of the formulated allicin based disinfectant is studied for its ant repelling property and its application on the vegetable *Brassica oleracea* (Cauliflower) inorder to improve its shelf life.

***Key words*:** Disinfectant, Allicin, Ant repellent, *Allium sativum, Brassica oleracea*

Pathogenic microorganisms such as bacteria, bacterial spores, fungi and viruses are responsible for the contamination of food and vegetables. The disinfectants and sanitizers which are currently being used contain chemical based antimicrobial agents. These synthetic disinfectants cause detrimental effects on both the environment and on human wellbeing. Synthetic disinfectants contain chlorine, ammonia, formaldehyde which are harmful to human health. The concept of formulating disinfectant involves the selection of simple natural compounds and formulating it to make it effective against contaminants [7].

The formulation of an allicin-based disinfectant offers a sustainable and multifunctional approach to hygiene management, pest control, and postharvest preservation. Allicin, the principal bioactive compound derived from garlic (*Allium sativum*), possesses strong antimicrobial, antioxidant, and insect-repelling properties that make it an effective natural substitute for synthetic disinfectants and pesticides [10]. In formulated form, stabilized allicin can act simultaneously as a disinfectant and a bio-repellent, providing a non-toxic means of deterring ants by disrupting their pheromone-based communication and foraging behavior through its characteristic sulfurous odor and volatile compounds. Beyond pest management, allicin exhibits significant preservative potential for *Brassica oleracea* species such as cabbage, broccoli, and cauliflower by inhibiting spoilage microorganisms like *Pseudomonas* and *Erwinia*, reducing physiological deterioration, and maintaining postharvest freshness. Its mechanism of action involves interaction with thiol groups in microbial enzymes, leading to metabolic inhibition and broad-spectrum antimicrobial effects. The incorporation of allicin in disinfectant formulations thus provides dual functionality ensuring microbial control and pest repellency while remaining biodegradable, residue-free, and safe for food systems and the environment [11]. Such innovation aligns with the growing demand for eco-friendly bioproducts in agriculture and food preservation, highlighting allicin's potential as a natural, green alternative to conventional chemical-based disinfectants.

MATERIALS AND METHODS

Allicin extraction

23g of skinned garlic cloves was added to 40mL of distilled water. It was crushed using blender for 10 seconds and left to rest for 10 minutes. Again, it was crushed using blender for 10 seconds. The trampled garlic was hard-pressed and the extract solution was filtered using cheesecloth. It was left overnight and centrifuged at 10,000 rpm for 10 minutes. The pellet was discarded and supernatant collected and stored at 4°C for further use [1].

Fig 1 Allicin extract

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Allicin characterization

Lead acetate test/ sulphur test

The collected extract (2 mL) was taken in a test tube and mixed with 3 - 4 drops of lead acetate. 2 - 3 drops of 40% Sodium hydroxide were added dropwise and mixed till the precipitate dissolved. The test tube containing the mixture was kept at boiling water bath for 2 mins and cooled.

Sodium nitroprusside test

The collected extract (2 mL) was taken in a test tube and 3 - 4 drops of sodium nitroprusside was added to it. The medium was tested for its alkalinity and adjusted by adding 2 - 3 drops of 40 % sodium hydroxide [2].

Isolation of saponin from coconut leaves

Coconut leaf samples were collected and were cut into small pieces. Small pieces were mashed using blender. 110g of blended samples were weighed and taken in a beaker. 500mL 0f 96% ethanol was added to sample and macerated for minimum 2 days. Ethanol was filtered and the filtrate was evaporated using magnetic stirrer. The evaporated samples were allowed to dry at room temperature overnight and saponins were obtained [3]. The extracted saponin which is to be used as surfactant in the disinfectant is characterized using FTIR (Fourier Transform Infrared Spectroscopy).

Fig 2 Surfactant from *Cocos nucifera*

Mint extract from peppermint leaves

Peppermint leaves were thoroughly washed and allowed to dry for 30 minutes at room temperature. The leaves samples were chopped to increase surface for contact while extracting using a suitable solvent. Accurately 60 g of fresh mint leaves was taken and placed in a 500 mL round bottom flask containing 250 mL of distilled water. The flask was fitted with a rubber stopper connected to a condenser and gradually the temperature was raised to 80 °C. At 100 °C it started boiling splitting off the essential oil from the sample substrate. The essential oil that was extracted from the mint sample along with the water vapour was collected in a receiver bottle. The condensate was cooled using water. The condensate was then transferred into a separating funnel forming two distinct layers with the oil at the top layer due to its lesser density compared to that of water. The tap of the separating funnel was opened to collect the water which was further used while the oil was separated [4].

Fig 3 Steam distillation of mint

Mint extract characterization

Benedict's test

2 mL mint extract was taken in test tube. 2 mL Benedict's reagent was added to sample. Then it was boiled for 2 min.

Ferric chloride test

2 mL of mint extract aqueous solution was taken in test tube. A few drops 5% ferric chloride solution was added.

Lead acetate test

The extract (2 mL) was taken in a test tube and mixed with 3 - 4 drops of lead acetate. 40 % Sodium hydroxide was added dropwise and mixed till the precipitate dissolved. The test tube was boiled for 2 mins and then cooled [5].

Formulation of disinfectant

A disinfectant formulation comprises of phenolic compound of natural origin; a surfactant sufficient to disperse the essential oil in an aqueous carrier; a suitable solvent, and sufficient water to make 100 weight percent. The method of formulating natural disinfectant, essentially involves the selection of available natural sources and enhancing its activity by formulating with active natural ingredients which have been discussed in (Table 1).

Table 1 Composition of natural disinfectant

Component	Natural source	Composition
Phenolic compound	Allicin	15 mL
Solvent	Mint extract	35 mL
Surfactant	Saponin	5 mL
Sequestering agent	Acetic acid	5 mL
Water	Water	L

Antimicrobial assay

The required glass wares and medium were sterilized at 121°C for 15 minutes. Test solution was prepared by adding 0.5 mL of *Bacillus subtilis* culture to 5 mL of prepared disinfectant solution and allowed for 10 minutes. Control solution was prepared by adding 0.5 mL culture in 5 mL distilled water and allowed for 10 minutes. Nutrient agar was poured in Petri plates and spread plate was done using test and control solution. The

plates were incubated at 37°C overnight and results were observed [6].

Case study on preservation of cauliflower

To extend the shelf life of cauliflower without compromising its nutritional quality the allicin based disinfectant can be used. The disinfectant when sprayed on cauliflower, prevents the discolouration on florets, maintains the texture and prevent it from pathogenic microorganisms [8].

To check the effectiveness of disinfectant on cauliflower the following tests are performed:

- pH monitoring
- Titratable acidity
- Sensory analysis
- Analysis of colour and weight

pH measurement

- 5g each of cauliflower without disinfectant (control) and cauliflower disinfected using allicin based disinfectant (test) were weighed using weighing balance.
- The weighed samples were crushed finely using mortar and pestle.
- Each crushed samples were suspended in 50 mL of distilled water and filtered.
- The pH meter was calibrated.
- Electrode of pH meter put in the control and test solution and the direct reading from pH meter was taken [8].
- pH of both control and test was noted for a period of 28 days in the interval of 4 days.

Titratable acidity

- 5g each of cauliflower without disinfectant (control) and cauliflower disinfected using allicin based disinfectant (test) were weighed using weighing balance.
- The weighed samples were crushed finely using mortar and pestle.
- Each crushed samples were suspended in 50 mL of distilled water and filtered.
- 20 mL of sample solution is taken in a conical flask and 3 to 4 drops of phenolphthalein was added.
- It was titrated against 0.1 N NaOH and end point was noted [16].

Titratable acidity was calculated using the formula:

$$\% \text{ Acidity} = \frac{N \times V \times M}{S \times 10}$$

- N-Normality of standard NaOH solution used for titration, V-Volume of standard NaOH used for titration, M- Molecular weight of the predominant acid in the sample divided by the number of hydrogen ions in the acid molecule that are titrated, S-Sample size [9].

Sensory analysis

Sensory quality of cauliflowers was evaluated at 6th and 7th day during storage. Cauliflower disinfected with allicin based disinfectant is the test and the cauliflower without disinfectant is control. The sensorial attributes of cauliflowers such as colour, odour, texture and overall satisfactoriness were evaluated by a panel of judges consisting of 15 scientific personnel and consumers including both males and females. Nine-point unstructured scale ranging from 1 (dislike extremely) to 9 (like extremely) was used to evaluate these sensory parameters [8].

Disinfectant as ant repellent

The ants are the most prevailing group of social insects belonging to the order Hymenoptera of class Insecta. Ants can defile and destroy some agricultural products and stored crops. They get attracted towards sweetness, food particles and function as a scavenger. Ant invasions are the annoyance of housekeepers. They can devastate plant crop and take over kitchens. Poisons and chemicals are effective, but are not safe to be used in the kitchen. The insecticidal properties of number of plant biomolecules have been discovered long ago. Botanical plant extracts are environmentally less harmful than synthetic pesticides in terms of controlling the pests. They possess biodegradability, broad spectrum of activity and the capability to reduce insect resistance. Synergistic effect of diverse plant compounds could facilitate in controlling the control pests. To control these insect pests, peoples are using synthetic insecticides which are toxic to non-target insects. The natural ant repellent plays an important role in earth's natural ecosystem [10].

The formulated disinfectant composed mainly of allicin not only contains the disinfecting and vegetable preserving properties but also exhibits ant repellent properties. The following case study shows the ant repelling properties of the disinfectant.

RESULTS AND DISCUSSION

Allicin

Lead acetate test/ sulphur test

There was appearance of brownish black precipitate. This confirms the presence of sulphur in prepared extract of allicin.

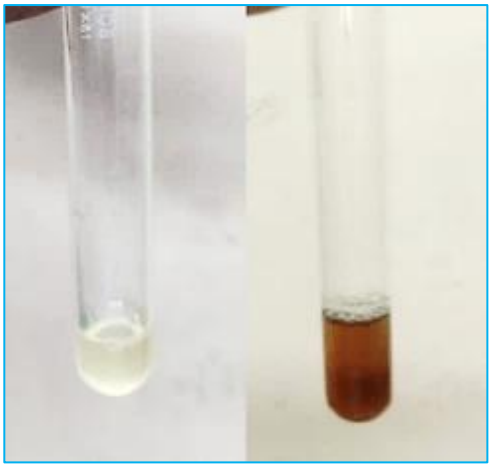

Fig 4 Allicin sample (control), Appearance of brownish black color (test)

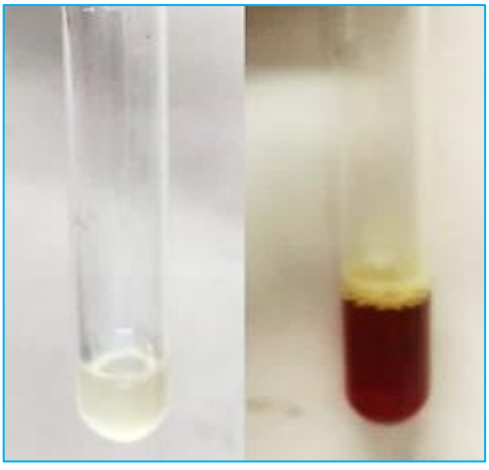

Fig 5 Allicin sample (control), Appearance of deep red color (test)

Sodium nitroprusside test

There was appearance of deep red colour indicating the presence of sulphur in given extract.

Characterization of saponin

Presence of saponin in coconut leaf extract is confirmed by FTIR. The FTIR spectra outcome of saponins is confirmed by the presence of functional groups OH, C=H, C=C and the finger print groups. To confirm the presence of crude saponin the broad infrared absorbance of the hydroxyl group (OH) should be at 3283.8 cm^{-1} and 3246.50 cm^{-1}. Carbon – hydrogen (C-H) absorption range should range between 2922.2 cm^{-1} – 2929.7 cm^{-1}. C-H absorption should be at 2923.23 cm^{-1}. There C=C absorbance for the samples should range between 1632.6cm^{-1} to 1636.3cm^{-1} [14].

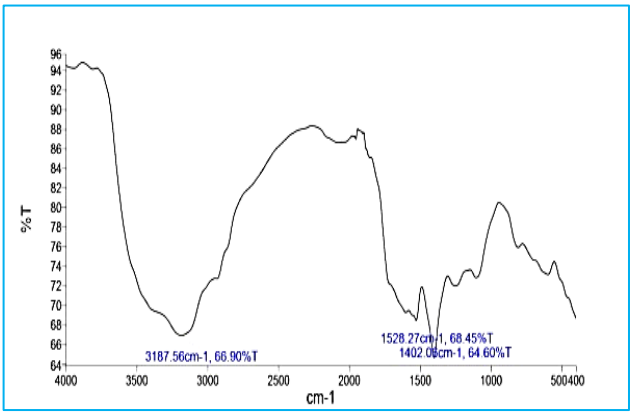

Fig 6 FTIR Spectrum for coconut leaf extract (saponin)

Interpretation: OH group was evident at 3187.56 cm^{-1}, CC stretching was recorded at 1528.27 cm^{-1} which confirms presence of saponins and CH deformations were observed at 1402.06 cm^{-1}.

Mint extract characterization

Benedict's test

Appearance of green colour indicates the presence of carbohydrates in the extracted sample.

Fig 7 Appearance of green colour indicating presence of carbohydrates

Fig 8 Appearance of dark green colour indicating presence of phenolic compounds

Fig 9 Appearance of brownish black colour

Ferric chloride test

Appearance of dark green colour which indicates the presence of phenolic compounds.

Lead acetate test

Appearance of brownish black colour which indicates the presence tannins and flavonoids.

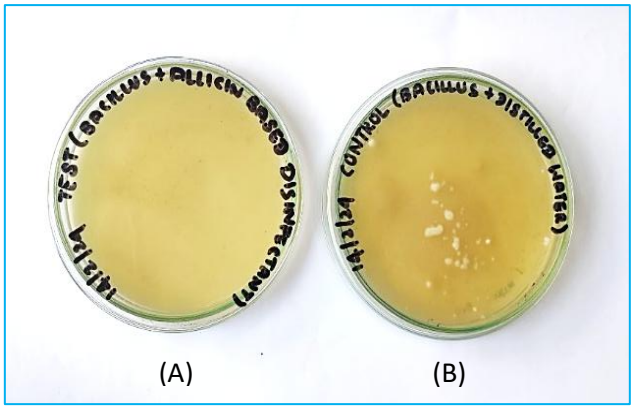

Fig 10 (A) Test sample subjected to disinfectant
(B) Control without disinfectant showing microbial growth

Antimicrobial assay

The effectiveness of disinfectant against microbes is determined by this method. Plating was done in two plates simultaneously. Control contains *Bacillus subtilis* whereas Test contains *Bacillus subtilis* and disinfectant. As we can observe from the (Fig 10) that the plate containing disinfectant does not show any microbial growth whereas the control plate without disinfectant shows microbial growth. From this we can say that the disinfectant has antimicrobial activity.

Table 2 pH values for control and test

Days	Control	Test
0	6.82	6.82
4	6.55	6.90
8	6.61	6.69
12	6.59	6.65
16	6.52	6.66
19	6.28	6.38
24	6.25	6.55
28	6.20	6.35

Case study on preservation of cauliflower

pH measurement

Decrease in the pH value indicates the spoilage of the vegetable. From the above graph we can infer that the pH of the control decreases day by day when compared to the test. This clearly shows that the disinfectant protects the vegetable from spoilage and maintains the pH.

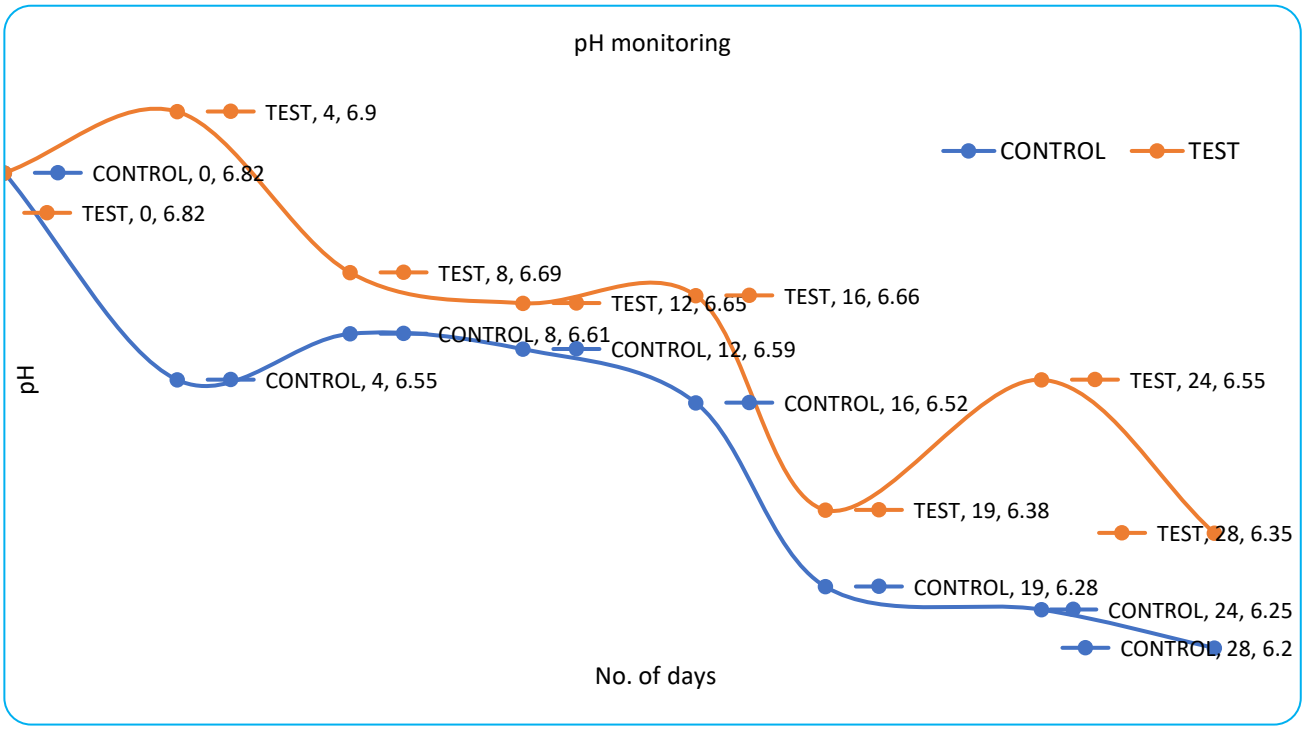

Fig 11 Graph for pH values of control and test against number of days

Table 3 Titre volume and titratable acidity of control and test

Days	Control		Test	
	Titre volume (mL)	Titratable acidity (%)	Titre volume (mL)	Titratable acidity (%)
0	0.6	0.015	0.6	0.015
4	0.9	0.023	0.7	0.017
8	0.9	0.023	0.7	0.017
12	0.9	0.023	0.8	0.021
16	0.9	0.023	0.8	0.021
19	1.6	0.041	1.2	0.031
24	2.4	0.061	1.3	0.033
28	2.8	0.074	1.4	0.036

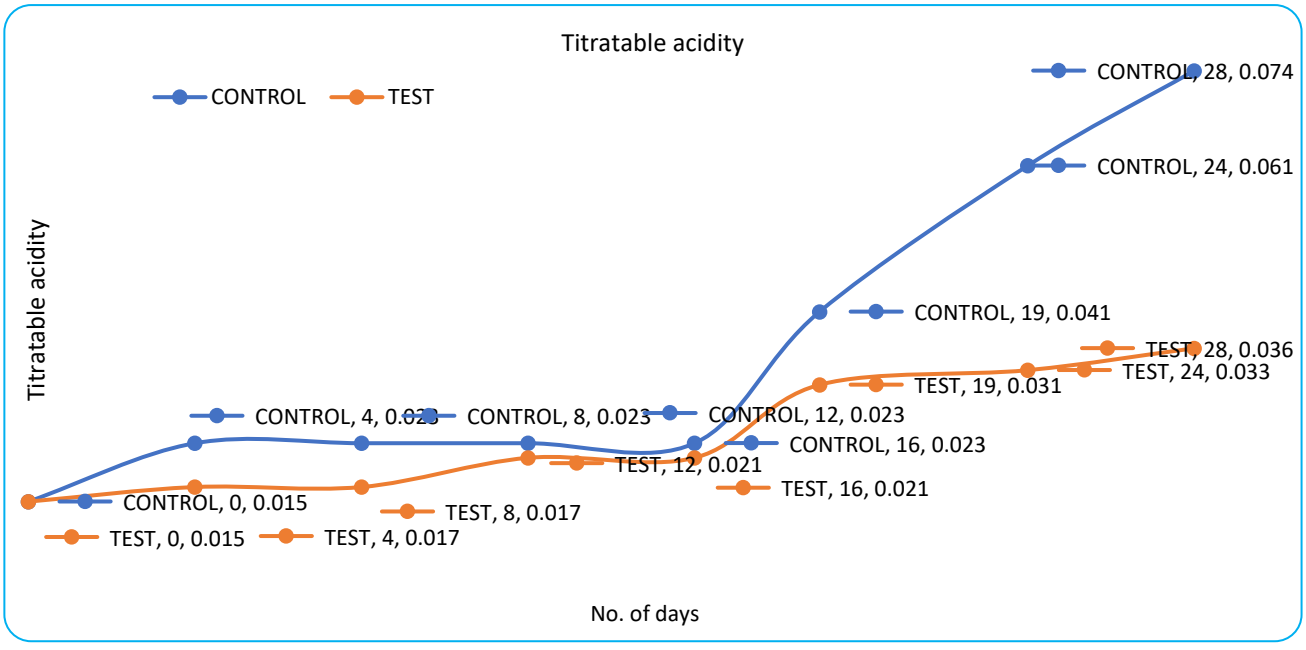

Fig 12 Graph for titratable acidity of control and test against number of days

Titratable acidity

Over a period of first 15 days the % titratable acidity of control and sample remains the same. After 15 days we can clearly observe that the acidity of control increases rapidly than that of the test cauliflower. Increasing in the acidity percentage indicates the spoilage of the vegetable. From the above graph we can infer that the acidity percentage of the control increases day by day when compared to the test. This clearly shows that the disinfectant protects the vegetable from spoilage and maintains the acidity.

Table 4 Average scores for sensory quality of control and test

Sensory quality	Scientific personnel		Consumers	
	Control	Test	Control	Test
Colour	3.8	6.9	5.1	7.9
Smell	5.4	6.2	4.6	7.6
Texture	5.5	7.4	4.1	8.5
Overall acceptability	4.9	6.8	4.6	8

Table 5 Percentage variability for sensory analysis

Percentage variability (%)	Scientific personnel	Consumers
	21.1	37.7

Sensory analysis

The control cauliflower has obtained an average score of 3.6 for colour, 5.4 for smell, 5.5 for texture with overall acceptability score of 4.9, whereas the test cauliflower sprayed with disinfectant obtained an average score of 6.9 for colour, 6.2 for smell, 7.4 for texture with overall acceptability score of 6.8 for which the scores have been awarded by a group of 10 scientific personnels. The control cauliflower has obtained an average score of 5.1 for colour, 4.6 for smell, 4.1 for texture with overall acceptability score of 4.6, whereas the test cauliflower sprayed with disinfectant obtained an average score of 7.9 for colour, 7.6 for smell, 8.5 for texture with overall acceptability score of 8 for which the scores have been awarded by a group of 10 consumers. By comparing the acceptability scores of both the cauliflower (test and control), the test cauliflower has more acceptable score when compared to control. This clearly shows that the disinfectant preserves the colour, smell, and texture of the cauliflower.

The percentage variability by scientific personnel and consumers were calculated and analysed. These values shows that the consumers have accepted the disinfected cauliflower as a healthy option because the difference in variability is more. The variability percentage by scientific personnel also shows a reasonable difference which can be accepted which are detailed in (Table 5).

Disinfectant as ant repellent

An ant colony containing numerous red ants have been identified and the formulated disinfectant was sprayed over them. Initially, there were several ants around the marked place but after an hour, the ants disappeared from that place. Ant colony was seen in (Fig 13). (Fig 14) depicts the ant colony after spraying allicin based disinfectant at zeroth time. From the (Fig 15) ants have been repelled from the marked place where the disinfectant was sprayed after an hour.

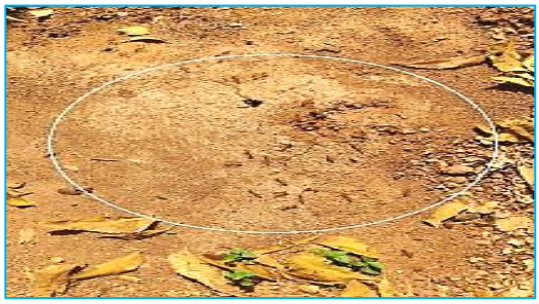

Fig 13 Ant colony before spraying disinfectant

Fig 14 Ant colony after spraying disinfectant

Fig 15 Ants repelled after 1 hour

The disinfectant contains ant repellent properties and has the ability to repel ants from a particular place within a specific time period without causing death of the ants.

CONCLUSION

The main focus on developing this disinfectant is for vegetable preservation. Disinfectants which are available in market contains toxic chemicals whereas this disinfectant was formulated using natural sources. The disinfectant was formulated using allicin, saponin, mint extract, acetic acid and water. The allicin was extracted from garlic using pressing extraction, saponin was extracted from coconut leaf and mint extract was extracted from mint by distillation. These products are completely non-toxic and are not harmful. The sources from which this disinfectant was developed is completely natural and environmentally friendly. Nowadays there are numerous

chemical preservation method for vegetables but they are harmful to human health. Using this allicin based disinfectant the vegetables can be preserved for long time and are non- toxic. The sources used for formulating natural disinfectant contains properties like antimicrobial, maintaining surface tension and contains various useful phytochemicals which have been identified by various characterisation techniques. To characterise allicin lead acetate test and sodium nitroprusside test was done to identify the presence of sulphur as it is an organo-sulphur compound. To identify the presence of saponin in coconut leaf extract FTIR was performed which confirmed the presence of saponin. The presence of useful phytochemicals which are present in mint extract are characterized by benedict's test for confirming the presence of carbohydrates, ferric chloride test for confirming the presence of phenolic compounds, and lead acetate test for confirming the presence of tannins and flavonoids. A case study on cauliflower clearly shows the potential of the disinfectant on vegetable preservation. The control and test cauliflower were monitored over 28 days and the parameters like weight, colour, pH and percentage acidity were noted. By interpreting those values, it can be understood that the test cauliflower where the disinfectant sprayed shows better readings and visuals. A microbial plating technique was also performed to identify the efficiency of disinfectant against microbes where the disinfectant inhibited the growth of microbes. From this we can also conclude that it has antimicrobial property. Another case study was also performed to check the ant repellent property of the disinfectant. When the disinfectant is sprayed over the ant colony within a specific time period, the ants disappeared. This property of the disinfectant also proves that it can be used to protect food and vegetables from insects.

Declaration of competing interest

The authors affirm that they have no known competing financial interests or personal relationships that could have come out to influence the work accounted in this paper.

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