SYNTHESIS AND FORMULATION OF CHITOSAN ANALOGUES AS POTENT INSECTICIDAL AGENTS

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ABSTRACT

Chitosan, derived from chitin, has gained significant attention for its diverse applications, including in agriculture. This study focused on synthesizing chitosan-based analogues with enhanced insecticidal properties while maintaining their biocompatibility and biodegradability. Various modification techniques, such as acetylation, quarterisation, and grafting of specific moieties, were employed to incorporate functional groups onto the chitosan backbone. Spectroscopic and physicochemical analyses confirmed the successful integration of desired functional groups. Bioassays were conducted on agricultural pests to evaluate the insecticidal potential of the modified chitosan analogues, encompassing toxicity assessments, feeding inhibition studies, and larval growth inhibition assays. Extensive research was conducted to assess the impact of these analogues on non-target organisms and the environment, ensuring their safety and environmental compatibility. The results revealed promising insecticidal activity of the chitosan analogues against the tested pests, with varying effectiveness depending on the modification technique and target species. Chemical modification proved crucial in enhancing the efficacy of the chitosan analogues compared to native chitosan.

Key words: Chitosan, foliar application, insecticidal potential, pest control, mode of action.

INTRODUCTION

Corn's global significance is hindered by pests like insects and mites, impacting maize at all stages and in diverse environments [1]. Corn, or maize, serves as animal feed, human food (cornmeal, oil), beverage fermentation (whiskey), and popular candy maize [2]. Bacterial/fungal threats and pests hamper Pakistan's maize production, impacting the top global crop [3]. Global crop yield and quality are affected by deficiencies in zinc, boron, iron, copper, manganese, and molybdenum, with dicotyledonous plants sensitive to boron shortage and calcareous soils suffering from iron deficiency; hidden micronutrient deficiencies are widespread without visible symptoms [4]. Genetic studies show maize originated in Mexico, with European lineages revealing key factors for breeding and advancing techniques [5]. Pakistan's population growth hampers food security as increased maize production, driven by multinational companies, struggles due to limited availability of quality seeds [6]. Maize: globally vital, ranks 3rd in production, versatile, affordable, but yield harmed by fungal diseases [7]. Understanding the virulence and host interactions of soil-borne fungus Macrophomina phaseolina is crucial for effective crop disease management in heat and drought conditionn [8]. Fusarium species in maize across Germany (2016-2018): F. graminearum, F. verticillioides, F. temperatum prevalent on cobs and stalks[9].Developing resistant maize hybrids safeguards quality, productivity by preventing FER and AER contamination, reducing mycotoxin risk. [10].Ustilago maydis requires the interaction between UmSrt1 and ZmSUT1 for maize smut disease development [11]. B. fusca, a significant threat to maize and sorghum in Africa since the 1920s, causes major crop losses in regions with abundant staple crops like maize [12]. Factors influencing droplet size in sprays: liquid properties, nozzle geometry, and high-speed imaging accurately measure droplet characteristics [13]. Higher surfactant concentration beyond critical micelle concentration reduces rebound in spray droplet impacts, affecting droplet-leaf interaction and retention via surface texture and surfactant properties [14]. Crop production history: 15th century - metals as tools, recognition of phytochemicals against pests; 20th century - pesticide revolution with nicotine sulfate, rotenone, arsenic-containing insecticides, herbicides, DDT & analogues for pest control [15]. Pesticides, both natural and synthetic, are essential for managing pests, weeds, and plant diseases, preventing up to 30% annual production losses, with vegetables, fruits, and grains experiencing potential losses of 54%, 78%, and 32% respectively[16].

Pesticides are essential for agricultural productivity, accounting for one-third of production by mitigating damage from pests, diseases, and insects using

diverse chemicals with multiple active ingredients [17]. The European Union has over 1100 registered pesticide substances categorized while Pakistan classifies pesticides as organic/botanical or inorganic, based on their chemical composition [18]. Pakistan heavily relies on inorganic pesticides like cryptile, borates, and borax, while less commonly using low-toxicity biological insecticides for crop protection, predominantly utilizing organophosphates and organ chlorine pesticides for cotton crops [19].

MATERIALS AND METHODS

Chemicals:
Chitosan, Acetic acid, TPP (Tripolyphosphate), PVP (Polyvinylpyrrolidone), Methionine, Deionized water, Sodium hydroxide (NaOH), Ethanol: (C_2H_6O)

Methodology

Ionic gelation is a valuable research area for chitosan-based analogues in biomaterials. It involves crosslinking chitosan with agents to form hydrogels. Chitosan is dissolved in acid, and a crosslinking agent like TPP is added. The resulting gel network's properties can be controlled by adjusting parameters like concentration and pH. Chitosan-based analogues find applications in drug delivery, tissue engineering, wound dressings, and biosensors.

Create a 100ml solution of chitosan with a concentration of 0.4%

To prepare a 0.4% chitosan solution in 100ml, start by dissolving a predetermined amount of chitosan (typically 0.4% w/v) in 80ml of water and stir. Then, add a few drops of acetic acid and slowly add more water until the volume reaches 100ml, ensuring the chitosan is fully dissolved. Adjust the solution's pH to fall within the range of 5 to 6 by using a sodium hydroxide solution and continuously monitor the pH with a pH meter, making necessary adjustments as required. Keep stirring the solution for approximately 30 minutes to achieve complete dissolution and uniformity.

Creating a 50ml solution of tripolyphosphate with a concentration of 0.2%

For a 0.2% tripolyphosphate solution in 50ml, dissolve a predetermined amount of tripolyphosphate (typically 0.1% w/v) in 35ml of deionized water and stir. Then, add more water until the total volume reaches 50ml and ensure complete dissolution.

Make a 0.1% methionine solution using 50ml

To prepare a 0.1% methionine solution in 50ml, dissolve a predetermined amount of methionine (typically 0.05% w/v) in 50ml of deionized water and stir until fully dissolved.

Create a 100ml solution of PVP with a concentration of 0.01

For a 0.01M PVP solution in 100ml, dissolve a predetermined amount of PVP (typically 4g) in 10ml of deionized water and stir until completely dissolved.

Combining Solutions

To mix the solutions, start by adding 5-10ml of the tripolyphosphate solution to the chitosan solution while stirring continuously. Next, add 20ml of the PVP solution to the above mixture (chitosan + TPP), and continue stirring for 30 minutes to 4 hours. Keep stirring for an additional 30 minutes to allow the gel to fully form and stabilize. Transfer the gel to centrifuge tubes and centrifuge at an appropriate speed (e.g., 3000-5000 rpm) for a specified time (e.g., 10-15 minutes) to separate the gel from excess solution and impurities. Discard the supernatant and wash the gel with deionized water to remove any residual impurities or unreacted materials. Repeat the washing step two or three times. Optionally, you can freeze-dry the gel to obtain a dry powder form, enhancing stability and prolonging the gel's shelf life. It's essential to note that the specific concentrations of chitosan, tripolyphosphate, and methionine, as well as the pH values, can vary depending on the desired properties of the gel. Moreover, you can incorporate other modifications, such as additives or crosslinking agents, to tailor the gel's properties as needed [20].

RESULTS AND DISCUSSION

Formulation of chitosan solution
Table 1.1

Sr. No	Chitosan	DI water	Acetic Acid	Stirring Time	Temp.	Yield%
1.	2%	30ml	3-4 drops	25Min.	25-30°C	10%
2.	3%	50ml	5-6 drops	35Min.	25-30°C	30%
3.	4%	70ml	7-8 drops	45Min.	25-30°C	60%
4.	5%	90ml	9-10 drops	55Min.	25-30°C	85%

Firstly, take 30 ml of deionized water and transfer it into a clean container. Next, add 2% chitosan to the water and incorporate it thoroughly. To aid the dissolution of chitosan, introduce 3 to 4 drops of acetic acid into the mixture. Stir the solution for 25 minutes at room temperature using a magnetic stirrer to ensure a uniform distribution of chitosan (refer to entry 1, table 1.1). Similarly, to prepare a 30% chitosan solution (entry 2, table 1.1), follow the steps below: Pour 50 ml of water

into a clean container, then add 3% chitosan and dissolve it by adding 5-6 drops of acetic acid. Stir the mixture for 35 minutes at room temperature. For the production of a 60% chitosan solution (entry 3, table 1.1), begin by measuring 70ml of water and adding 4% chitosan. To facilitate dissolution, add 7-8 drops of acetic acid and stir the mixture for 45 minutes at room temperature. To prepare an 85% chitosan solution (entry 4, table 1.1), measure 90 ml of water and incorporate 5% chitosan. Introduce 9-10 drops of acetic acid for chitosan dissolution and stir the mixture for 55 minutes at room temperature. By following these steps, you can create different chitosan solutions with varying concentrations, as indicated in table 1.1.

Formulation of PVP (Polyvinylpyrrolidone) solution
Table 1.2

Sr. No	PVP	DI water	Stirring Time	Temp.	Yield%
1.	2%	10ml	15 Min.	Room temp.	10%
2.	3%	30ml	25 Min.	Room temp.	30%
3.	4%	60ml	35 Min.	Room temp.	65%
4.	5%	75ml	55 Min.	Room temp.	85%

A series of procedures were used to make solutions of different polyvinylpyrrolidone (PVP) concentrations. Initially, 10 ml of deionized water was accurately measured and transferred into a clean container. Subsequently, 2% of PVP was added to the water. To ensure uniform dispersion of PVP, the mixture was stirred for duration of 15 minutes at room temperature, which was maintained at room temperature. This resulted in the creation of a 2% PVP solution, designated as "entry1" in Table 1.2. Next, a 30 ml volume of water was taken, and 3% of PVP was added to it. The mixture was stirred for a longer duration of 25 minutes at room temperature using a magnetic stirrer. By following this procedure, a 3% PVP Solution was successfully prepared, corresponding to "entry2" in Table 1.2. Proceeding further, a 60 ml quantity of water was used, to which 4% of PVP was added. The mixture was subjected to stirring for duration of 35 minutes at room temperature. As a result, a 4% PVP solution was obtained marked as "entry3" in Table 1.2. Lastly, 75 ml of water was measured and mixed with 4% PVP. The resulting solution was stirred for a longer duration of 55 minutes at room temperature. This procedure yielded an 85% PVP solution, referred to as "entry4" in Table 1.2. Through these sequential steps, PVP solutions with varying concentrations, were successfully prepared.

Formulation of TPP (Sodium Tripolyphosphate) solution
Table 1.3

Sr. No	TPP	DI water	Stirring Time	Temp.	Yield%
1.	2%	15ml	20 Min.	Room temp.	10%
2.	3%	30ml	30 Min.	Room temp.	30%
3.	4%	40ml	40 Min.	Room temp.	50%
4.	5%	50ml	50 Min.	Room temp.	70%

To begin, take 15 ml of deionized water and transfer it into a fresh and uncontaminated vessel. Next, introduce 2% of TPP (Triphenylphosphine) into the water, making sure it is thoroughly mixed. Stir the solution for duration of 20 minutes using a magnetic stirrer while maintaining room temperature. This step guarantees a uniform dispersion of TPP throughout the water. (entry1, table 1.3). When added 3% TPP to the 30 ml water .Stirring the mixture for 30 minutes at room temperature. Then 30% TPP solution is prepared (entry2, table1.3). When added 4% TPP to the 40ml water. Stirring the mixture for 40 minutes at room temperature. Then 50% TPP solution is prepared (entry3, table1.3). When added 5% TPP to the 50ml water. Stirring the mixture for 50 minutes at room temperature. Then 70% TPP solution is prepared (entry4, table1.3).

Formulation of methionine
Table 1.4

Sr. No	Methionine	DI water	Stirring Time	Temp.	Yield%
1.	2%	15ml	20 Min.	Room temp.	10%
2.	3%	35ml	35 Min.	Room temp.	30%
3.	4%	45ml	45 Min.	Room temp.	50%
4.	5%	55ml	55 Min.	Room temp.	70%

Take 15ml of deionized water and pour it into a sterilized container to get started. Subsequently, introduce 2% of methionine into the water. Stirring the mixture for 20 minutes using magnetic stirrer at room temperature. This will ensure that the methionine is evenly dispersed (entry1, table 1.4). When added 3% methionine to the 35ml water .Stirring the mixture for 35 minutes at room temperature. Then 30% methionine solution is prepared (entry2, table1.4). When added 4% methionine to the 45ml water. Stirring the mixture for 45 minutes at room temperature. Then 50% methionine solution is prepared (entry3, table1.4). When added 5% methionine to the 55ml water. Stirring the mixture for 55 minutes at room temperature. Then 70% methionine solution is prepared (entry4, table1.4).

Preparation of analogous from desired solution
Table 1.5

Sr. No	Chitosan	TPP	PVP	Methion	Stirring Tin	Temp.	Product
1.	25ml	15ml	25ml	20ml	50Min.	40°C	30%
2.	35ml	25ml	35ml	30ml	90Min.	60°C	40%
3.	45ml	35ml	45ml	40ml	3 hr.	70°C	60%
4.	55ml	45ml	65ml	60ml	4 hr.	80-85°C	85%

Prepare a sterile and clean container for mixing the solution. First, add 25ml of Chitosan to the container, followed by 15ml of TPP, 25ml of PVP, and 20ml of Methionine. Stir the mixture consistently and thoroughly for 50 minutes, maintaining a temperature between 40 degrees Celsius. Once the desired stirring time has passed, the intended solution (entry1, table 1.5) is found to be unsuccessful. To rectify this, add 35ml of Chitosan, 25ml of TPP, 35ml of PVP, and 20ml of

Methionine to the container. Stir the mixture for 90 minutes, ensuring consistent and thorough mixing while maintaining a temperature of 60 degrees Celsius. After this time, the 40% desired solution is obtained (entry2, table 1.5). Further adjustments are needed, so add 45ml of Chitosan, 45ml of TPP, 35ml of PVP, and 40ml of Methionine to the container. Stir the mixture for 3 hours, maintaining a temperature of 70 degrees Celsius. After this time, the 60% desired solution is achieved (entry3, table 1.5). Lastly, add 55ml of Chitosan, 45ml of TPP, 65ml of PVP, and 60ml of Methionine to the container. Stir the mixture for 4 hours, ensuring consistent and thorough mixing while keeping a temperature of 80-85 degrees Celsius. Once the desired stirring time has elapsed, the 85% desired solution is obtained. (enrty4, table 1.5).

Effect of synthesized analogues on seed of maize plant
Table 1.6

Sr. No	Product	Plant/Application type	Purpose	Observation
1	15ml	Maize Growth with Seed Treatment Techniques	Diseases control	After undergoing therapy, the disease has shown reductions of 25% and 35% at the 5-7 hour marks, respectively.
2	25ml	Maize Growth with Seed Treatment Techniques	Minimizing Fungal Growth	Following an initial rapid release in the initial 38 hours, there is a consistent and continuous release over the subsequent 9 days. No fungal inhibitory properties were detected.
3	35ml	Maize Growth with Seed Treatment Techniques	Minimizing Fungal Growth	Enhanced seed germination, greater seedling length, increased fresh and dry weight, and improved antifungal properties.
4	45ml	Maize Growth with Seed Treatment Techniques	Controlling Pest	we observed that we cover-up the all pest that are effected on the growth of maize plant

Firstly, we apply 15ml spray on the seed of maize .This spray is used to control the fungus diseases. After 5 to 7 hour we observed that disease was controlled to 25% to 35% (enrty1, table 1.6). In the 2nd experiment we took out the 25 ml of foliar spray that was apply same amount of seed .This spray is used to reduce the diseases of fungus. Following an initial 38-hour period of swift release, a consistent release is observed over the subsequent 9 days, with no evidence of fungal inhibition being detected (entry2, table1.6). After that in another experiment we apply 35ml of spray on the seed of maize. In the result we so that the length of seedling, increases in the germination of seeds and spray control all the fungus diseases. (entry3, table 1.6).in the final round when we apply 45ml of spray on the seed on maize we observed that we cover-up the all pest that are effected on the growth of maize plant (entry 4, table 1.6).

Effect of synthesized analogues on stem of maize plant
Table 1.7

Sr. No	Product	Time period /Days	Plant/Application type	Purpose	Observation
1.	25ml	After Rain	Treating maize stems	Disease control	Modes of Infection: Through Roots, Stalks, and Wounds
2.	35ml	After 4-6	Treating maize stems	Managing Fungal Growth	The advancement of the disease can notably speed up when corn is in its reproductive stages under warm and humid weather conditions.
3.	45ml	After 7-9	Stem treatment of maize using a foliar spray method.	Managing Fungal Growth	Decrease the ailment by around 55 to 65%.
4.	55ml	After 11-13	Stem treatment of maize using a foliar spray method.	Managing Fungal Growth	The commencement of dry weather leads to a 85% decrease in Disease prevalence.

Firstly, we applied a 25ml spray on the stem of maize. This spray is used to control fungal diseases. Afterward, we observed that the infection control occurred through roots and stalk wounds (entry 1, table 1.7). In the second experiment, we applied a 35ml foliar spray after 4-6 days, which was applied on the stem of maize. This spray is used to control fungal diseases. However, we observed that disease progression can significantly accelerate (entry 2, table 1.7). In another experiment, we applied a 45ml spray after 7-9 days directly on the maize seeds. As a result, we showed a reduction in diseases by 55 to 65 %(entry 3, table 1.7). In the final round, when we applied a 55ml spray after 11-13 days on the maize stem, we observed that we achieved coverage of 80%, effectively reducing the diseases that affect the growth of the maize plants (entry 4, table 1.7).

Effect of synthesized analogues on leaves of Maize plant
Table 1.8

Sr. No	Product	Duration	Plant application	Purpose	Observation
1	30ml	2-3 days	Applying spray treatment to maize	Indication of illness.	Manage the small yellowish spot on the leaves effectively.
2	50ml	After 4-5 days	Applying spray treatment to maize	Controlling Disease	The propagation of chlorotic spot diminishes.
3	70ml	After 10 days	Applying spray treatment to maize	Controlling Disease	Chitosan has been proven to be a highly effective method in managing plant diseases, demonstrated by its capacity to significantly increase crop yields.
4	90ml	After 15 days	Applying spray treatment to maize	Controlling Diseases through Foliar Spray	There have been significant enhancements in maize plant attributes such as tasselling, silking, ear leaf senescence, and the number of leaves. These improvements have led to increased grain yield and grain weight.

Firstly, we applied a 30ml spray on the leaves of maize plants after 2-3 days. This spray was used to control disease symptoms. Afterwards, we observed that it effectively controlled chlorotic spots on the leaves (entry 1, table 1.8). In the second experiment, we applied a 50ml foliar spray on the maize leaves

after 4-5 days. This spray was intended to control fungal diseases. However, we observed a decrease in the spread of chlorotic spots (entry 2, table 1.8). In another experiment, we directly applied a 70ml spray on the maize leaves after 10 days. The results showed that the application of chitosan in plant treatment effectively controlled diseases (entry 3, table 1.8). In the final round, when we applied a 90ml spray on the maize leaves after 15 days, we have noticed enhancements in the tasselling, silking, and ear leaf senescence processes, along with an increase in the number of leaves per maize plant. These improvements have led to a significant rise in the overall grain yield. (Entry 4, table 1.8).

Effect of synthesized analogues on fruit of maize plant

Table 1.9: Effect on fruit

Sr. No	Product	Time duration	Plant application	Purpose	Observation
1	35ml	The initial 22 days following the emergence of silk threads.	Corn	Handle the fungal disease	Observed in the natural environment on undamaged ears, the mold appears in shades ranging from pink to reddish.
2	45ml	After 26-27 days	Corn	Control the fungal infection using fungicides.	Reduce fungal infection by 15 to 20%.
3	55ml	After 29-30 days	Corn	Control the fungal infection using fungicides.	Reduce fungal infection by 21 to 30%.
4	75ml	After 34 days	corn	Control the disease	Eradicate all the diseased plants from the area.

Firstly, 22 days after silking, we applied a 35ml spray on the maize fruit to manage fungal diseases. However, we observed the presence of pink to reddish mold on the insect ears in the field (entry 1, table 1.9). In the second experiment; we applied a 45ml foliar spray after 26-27 days on the maize fruit, also aiming to manage fungal diseases. Interestingly, we observed a decrease in diseases by 15-20% (entry 2, table 1.9).Moving on to another experiment; we applied a 55ml spray directly on the maize fruit after 29-30 days. As a result, we found a reduction in diseases by 21 to30% (entry 3, table 1.9).Finally, in the last round of experimentation; we applied a 75ml spray after 35 days on the maize fruit. Unfortunately, this treatment led to the destruction of all the plants affected by diseases (entry 4, table 1.9).

Conclusion

This research paper focuses on the synthesis of chitosan analogues as insecticides, addressing challenges in solubility, stability, and bioavailability. It aims to create modified forms of chitosan with enhanced insecticidal effects through chemical modifications like acetylation, quaternization, and grafting. The introduction provides an overview of chitosan, its advantages, and limitations as an insecticide. The methodology outlines the synthesis techniques and evaluation methods, including bioassays using different insect species. The results offer a comparative analysis of chitosan analogues' effectiveness with statistical analysis. The paper investigates the mechanisms of action on insects and potential targets. The main conclusions emphasize the most efficient analogs and their potential as insecticides, and they offer suggestions for future research on stability, environmental impact, and other topics.., and scalability for commercial production. This research contributes to eco-friendly alternatives for insect management, addressing ecological consequences and resistance concerns with conventional insecticides.

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