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RESEARCH ARTICLE

Antifoliary activity of gold nanoparticles against *Rhizophora apiculata* saplings.

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Abstract

Application of safe nano-coating has become a topic of great interest in the field of bio-nanotechnology because of their potential for increased shelf life of many bio-based products. The present study investigated the synthesis of gold nanoparticles and its antifoliary activity against *Rhizophora apiculata* saplings. The gold nanoparticles sprayed in the *Rhizophora* saplings showed good antifoliary activity against the leaf eating insects as well as growth enhancement of the saplings. In the present study, after 30 days of monitoring, the nanoparticle increased the shelf life of treated plants when compared to control with respect to height, internodal difference, leaf area and number of leaves. This study highlighted the possibility of using gold nanoparticles as an antifoliar agent to prevent medicinal, endangered and rare plants of coastal origin.

Introduction:-

The major challenge in agro-based industries is to keep the plants, fruits and vegetables safe and clean until they reach the consumers hands. The natural coating of plants, fruits and vegetables is not adequate to offer safety against water loss and high respiration rate, thus resulting in loss of weight and nutrient during storage. The U.S. Centers for Disease Control and Prevention estimates around 76 million cases of food borne illness in the United States each year, resulting in about 5000 deaths (Mead et al 1999).

The main principles of spraying antifoliar agent in plants, is depend on the characteristics of the coating materials used for their preparation. Nowadays, the primary spraying material used in plants is hormones and fertilizers. Normally, hormones are good growth barriers, but they give slight resistance to insects and microbes. In difference, biopolymer-based films are often good barriers to oxygen and carbon dioxide, but they offer little protection against moisture migration (Jochenweiss and McClements, 2006). Coatings or films could serve as moisture, lipid, and gas barriers. On the other hand, they could improve the textural properties of plants or serve as carriers of functional agents such as colours, flavours, antioxidants, nutrients, and antimicrobials. Various microbes are responsible for the spoilage of plants, thus decreasing their quality and shelf life.

The antifoliary coating process without the use of commercially used cost effective hormone and insecticidal agent has been playing more vital role in controlling and preventing insecticidal and microbial outbreaks. Nowadays a variety of commercial pesticide and insecticides are available (Davidson, 2001). The major disadvantages of these agents are their lower stability and activity unless maintained at their optimum conditions (pH and temperature).

Application of safe nano-coating and suitable packaging has become a topic of great interest in the field of food nanotechnology because of their potential for increased shelf life of many food products (Ahvenainen, 2003). Over the past decade, there has been a strong push towards the development of silver and gold-containing materials for commercial use that exhibit antimicrobial or bactericidal properties. Silver and gold nanoparticles have a broad spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria, and there is also minimal development of bacterial resistance (Ip *et al.,* 2006). Because chemical synthesis methods produce a toxic substance as by-product, there is a big challenge to develop a new protocol that is a reliable, green chemistry process for the synthesis of nanoparticles that does not use toxic chemicals in the synthesis protocols.

In the present study, the gold nanoparticles, synthesized from the mangrove extract (*Avicennia marina*) were sprayed in Rhizophora saplings and they were tested for antifoliary activity.

Materials and methods:-

Synthesis of gold nanoparticle solution :-

For synthesis of gold nanoparticles, 0.1mM of 90 ml of chloro auric acid solution was mixed with 10 ml of leaf extract of Avicennia marina in a 250 ml Erlenmeyer flask and incubated at 25°C in dark. After the visual observation of colour change, the solution was centrifuged for 1 hour at 10,000 rpm. Then the supernatant was discarded. Again the sample was centrifuged at 10,000 rpm for 1 hour. And the supernatant was saved; it was used for further experiments.

Preparation of gold nanoparticles :-

For nanoparticle preparation, 100 ml of nanoparticle solution was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 *rpm* for 60 *min* and the pellet was obtained. This was followed by re-dispersion of the pellet of nanoparticles into 1 ml of deionized water. Thereafter, the purified suspension was freeze-dried to obtain dried powder. Finally, the dried nanoparticles were vortexed with deionized water.

Application of gold nanopartricles to *Rhizophora apiculata* saplings

Saplings of *Rhizophora apiculata* were placed in mangrove nursery, located in CAS in marine biology, Annamalai university, Parangipettai. Twelve saplings of 3 sets [Each set containing 4 plants- Contro1 (untreated) Control2 (treated) Test1 (untreated) Test2 (treated)] were labelled and kept in poly bags. The saplings of *R.apiculata* were sprayed with gold nanoparticles for every two days interval up to 30 days. Every two days interval, the height, number of leaves, internodal difference was noted for each treatment. After 1 month, the leaf area was calculated for one leaf from each sapling.

Results:-

The plant extract incubated with choloro auric acid, at the beginning of the reaction showed straw yellow colour, and gradually increased in colour intensity to ruby red, with the increasing period of incubation (Fig.1.). The colour of the reaction mixture changed to intense ruby red after 4 hours of incubation. Control without plant extract did not exhibit any colour change. (Asmathunisha et al 2010, Kathiresan et al 2010, Asmathunisha and Kathiresan 2013).

Fig.1. Synthesis of gold nanoparticles using Avicennia marina

The sets of plants with poly bags were shown in Fig2. The saplings treated with gold nanoparticles exihibited very less infected leaves and very fast growth (Table1 & Figure 3). Among all parameters tested, the number of leaves (100%) was more pronounced followed by height(30.95%). The saplings untreated with gold nanoparticles exhibited moderate growth (Table2 & Figure 3). Among all parameters tested the internodal difference was more pronounced followed by leaf area.

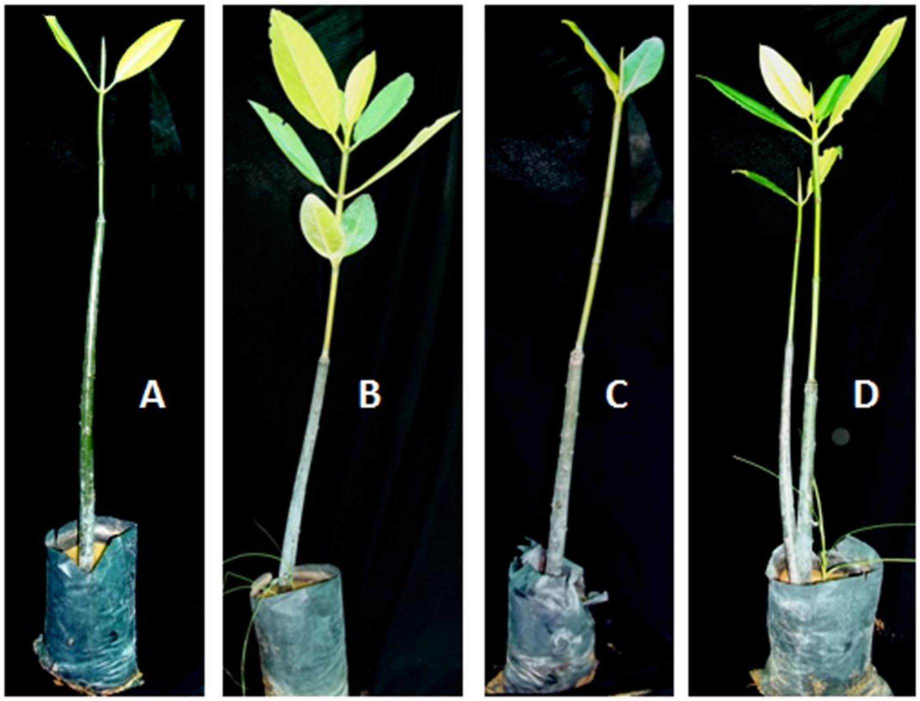

Fig. 2. ***Rhizophora apiculata*** **saplings used for treatments**

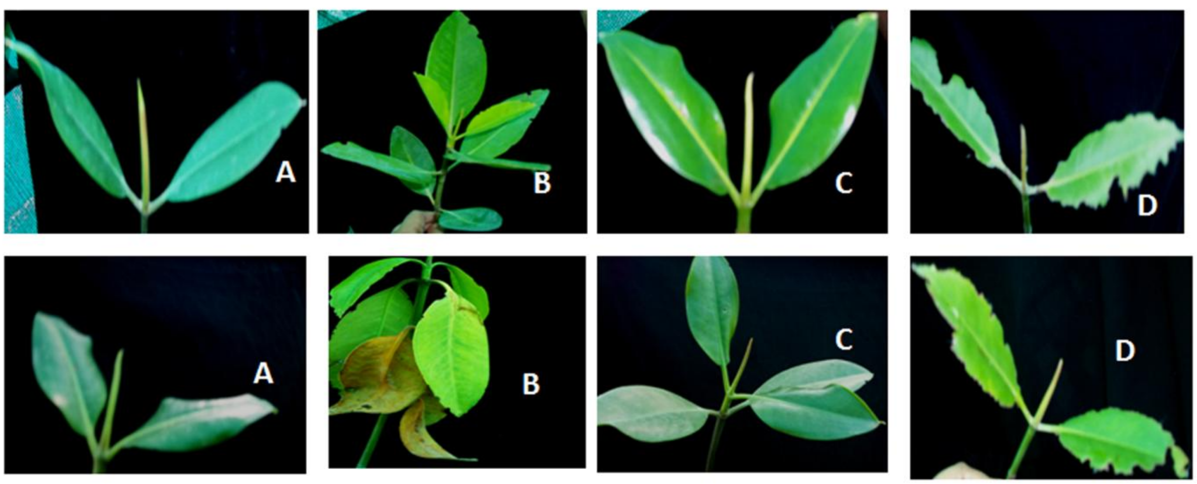

Fig. 3. (A& B) Untreated Plants (C&D) Treated plants – Before and After treatments

Table. 1.Physical parameters analysed in Rhizophora saplings treated with gold nanoparticles

Treated plants	Height(cm)			Inter nodal diff(cm)			Leaf area(cm^2)			No of leaves per plant		
	0day	30day	%Increase	0day	30day	%increase	0day	30day	%increase	0day	30day	%increase
Normal plant	48	57	18.75	7.2	9	25	47	54	14.89	2	4	100
Affected plant	42	55	30.95	8.3	10.3	24.09	54	61	12.96	4	4	0

Table 2. Physical parameters analysed in untreated Rhizophora saplings

UnTreated plants	Height(cm)			Inter nodal diff(cm)			Leaf area(cm^2)			No of leaves per plant		
	0day	30day	%increase	0day	30day	%increase	0day	30day	%increase	0day	30day	%Increase
Normal plant	51	55	7.84	4.1	5	21.95	42	49	16.66	2	2	0
Affected plant	39	43	10.25	8.5	9.4	10.58	38	30	-21	6	4	-33.3

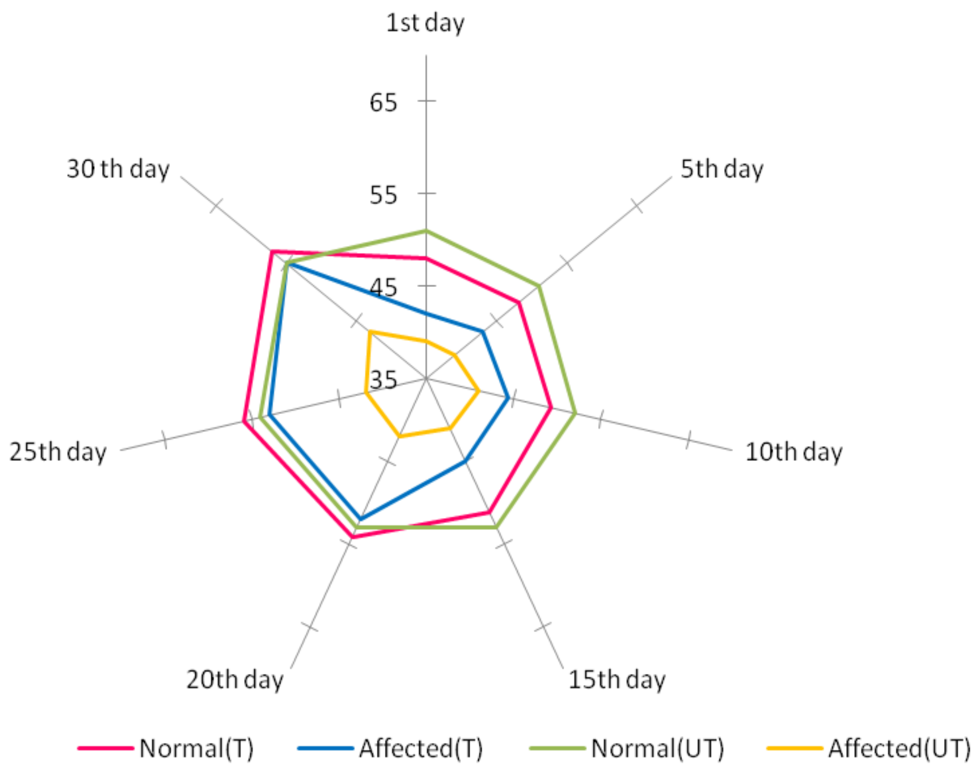

Fig.4. Analysis of Height in treated saplings

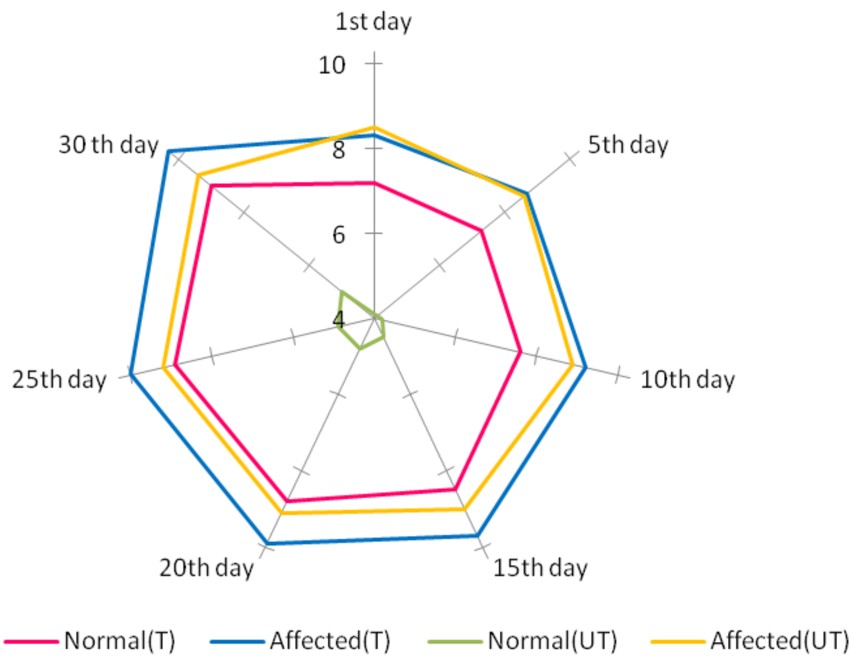

Fig.5. Analysis of internodal difference in treated saplings

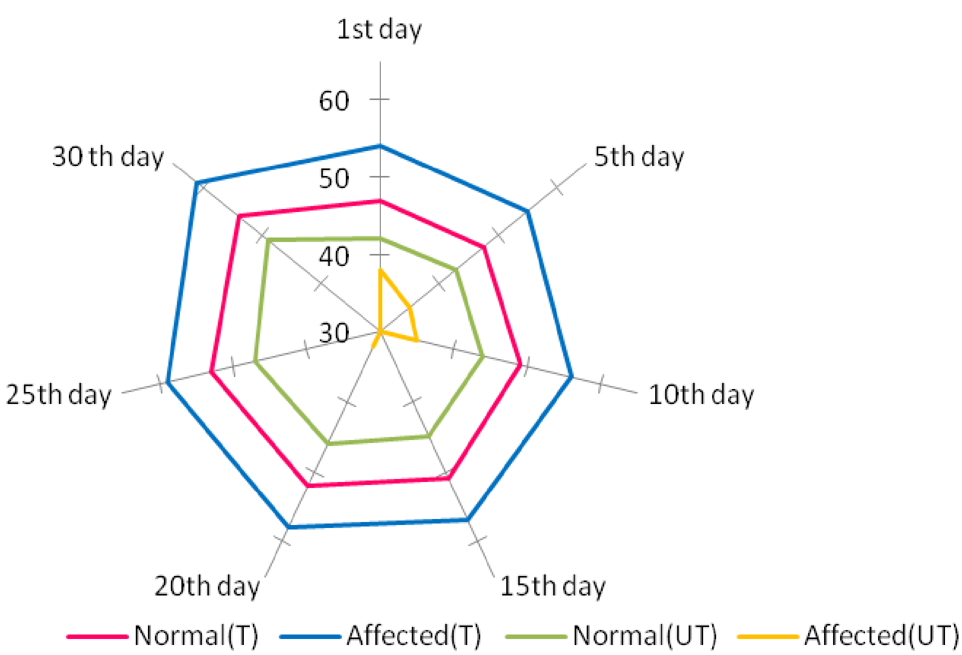

Fig.6. Analysis of leaf area in treated saplings

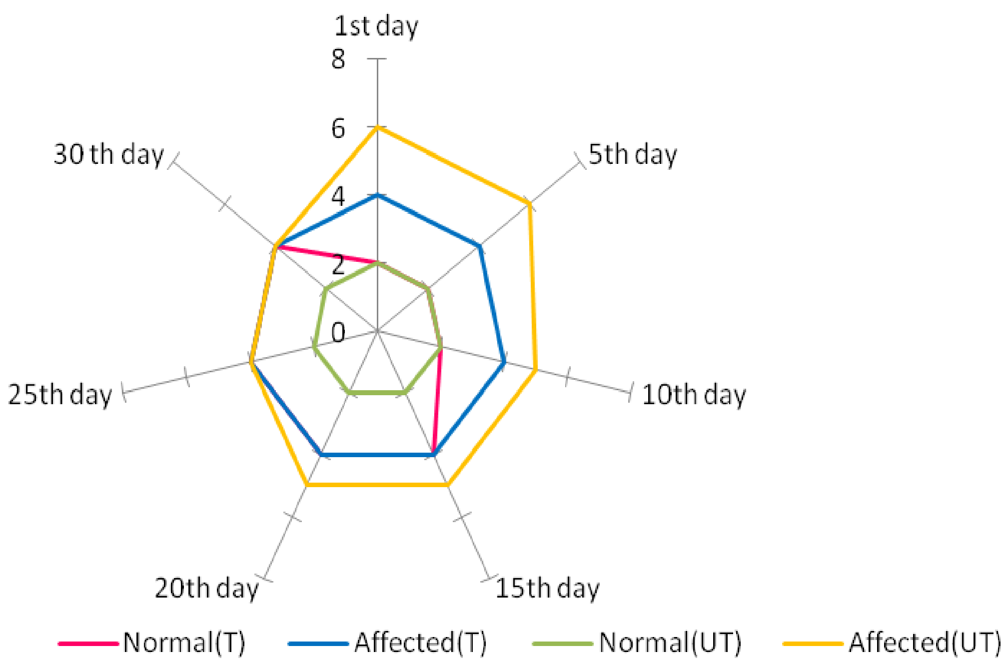

Fig. 7. Analysis of number of leaves in treated saplings

Discussion:-

The materials used in nano size propose improved functionality over traditional composites in terms of barrier properties, strength, elasticity and optical clarity. Nanocomposites can be functionalised to include other characteristics. For example, antimicrobial properties, visual indicators of food freshness, means of identification and authentication, and approaches to augment the ease of tracking (Robinson and Morinson, 2010). In the present study, *Rhizophora* saplings were sprayed using nano particles and the plants were monitored for antifoliary activity. There was a drastic increase in antifoliary activity for the first 15 days, and then got stabilized and increased relatively slowly, until the 30th day.

The gold nanoparticle sprayed with *Rhizophora* saplings showed good antifoliary activity against the leaf eating insects as well as it increase the growth of the saplings. In the present study, after 15 days of monitoring, the nanoparticle increased the shelf life of treated plants when compared to control with respect to height, intermodal difference, leaf area and number of leaves (Fig. 4 -7). When compared to untreated normal plant (7.84%), the height of the normal plant treated with gold nanoparticles increased up to 18.75%. Similarly, the height of the affected plant treated with gold nanoparticles showed 30.95% increase than untreated affect plant (10.25%). The difference of the internodes drastically increased in the treatment of gold nanoparticles in normal plant (25%) than affected plants (24%). The leaf area is the main physical parameters analyzed in foliar treatments. When compared to untreated affected plant (-21%), the surface area of the affected plant treated with gold nanoparticles increased up to 12.96%. The number of leaves in gold nanoparticles treated affected plants remains same compare to untreated affected plants. The untreated plants shed 2 leaves after 30 days of treatment. These results showed gold nanoparticles sprayed in *Rhizophora* saplings are suitable for prevention against foliary.

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