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*Research Article*

# Integration between Compost, *Trichoderma harzianum* and Essential Oils for Controlling Peanut Crown Rot under Field Conditions

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The effect of *T. harzianum* and some essential oils alone or in combination with compost on the peanut crown rot disease under field conditions was evaluated. Under laboratory conditions, results indicated that all concentrations of essential oils significantly reduced the growth of *A. niger*. Complete reduction was obtained with thyme and lemongrass oils at 0.5%. All *T. harzianum* isolates significantly reduced the growth of *A. niger*. The highest reduction was obtained with isolate no. 1 which reduced the growth by 81.1%. Under field conditions, results indicated that all treatments significantly reduced the peanut crown rot disease. The highest reduction was obtained with combined treatments (compost + *T. harzianum* + thyme and compost + *T. harzianum* + lemongrass) which reduced the disease incidence at both pre- and post-emergence growth stages, respectively. Similar trend corresponding to the previous treatments significantly increased the peanut yield which calculated as an increase in yield more than 75.0 and 80.0 during two growing seasons, respectively. It could be suggested that combined treatment between biocompost and essential oils might be used commercially for controlling peanut crown rot disease under field conditions.

## 1. Introduction

Peanut (*Arachis hypogaea* L.) is attacked by certain soil borne fungi causing root diseases. The main pathogen responsible for crown rot incidence of peanut was reported to be *Aspergillus niger* [1]. Crown rot of peanut was found in the sandy and reclaimed soils in Egypt [2, 3]. Also, it was reported that the fungus *Aspergillus niger* may cause seed rot and preemergence dampingoff of seedlings; the most obvious symptom is the sudden wilting of young plants [4–6]. The economic importance of Aspergillus crown rot is difficult to assess. Generally scattered plants are affected, although stand losses of 50% have been reported in isolated fields [7]. In Egypt, due to the economic importance of peanut, the farmers repeat planting even in the same land, which leads to a high buildup of pathogens, causing serious losses that could reach up to 18% [3]. As the management strategy followed by the farmers was considered to be an unwise, intensive use of fungicides, this strategy was not a satisfactory solution for controlling root rot disease. An investigation of crown rot disease is considered particularly important in light of its wide prevalence in Egypt, particularly in sandy soils. The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries [8]. *Trichoderma* spp. are effective biocontrol agents for a number of soil-borne plant pathogens and also known for their ability to enhance plant growth [9, 10]. Recently, it was suggested that *Trichoderma* affects induced systemic resistance (ISR) mechanism in plants [11–13]. On the other hand, the incidence of several soil-borne plant pathogens has also been reduced by using composts made of different raw materials [14–18]. *Trichoderma,* in combination with composts from agricultural wastes, was used to suppress *Rhizoctonia solani* in cucumber seedlings [19], and *Trichoderma* sp. and sewage sludge compost were used to suppress Fusarium wilt of tomato [17]. Currently, it is believed that a combination of antagonistic microbes with mature compost may be more efficient in inhibiting disease than using single antagonistic microbial strains or compost alone [8, 17, 19].

The objective of the present work was designed to evaluate the effect of *T. harzianum* and some essential oils alone or in combination with compost on the peanut crown rot disease under field conditions.

## 2. Materials and Methods

*2.1. Sources of Pathogenic Fungi, Bioagents, Peanut Seeds, and Compost.* Pathogenic fungal isolate *Aspergillus niger*, as the causal agent of peanut crown rot in addition to the bioagent *T. harzianum,* was kindly obtained from Plant Pathology Department, National Research Centre, Giza, Egypt. Meanwhile, peanut seeds cv. Giza 6 were obtained from Oil Crops Research Deptartment, Agricultural Research Centre, Giza, Egypt. Compost made from agricultural wastes was obtained from El-Nile Company, Egypt. Commercial essential oils of thyme (*a.i.* thymol, 60%); rose (*a.i.* citronellol 33%); and lemongrass (*a.i.* 85% citral) were used in the present work. Essential oils used in the study were obtained from Chemical Industrial Development Company (CID), Egypt. The essential oils were stored in dark glass bottles at 4°C until use.

*2.2. Laboratory Tests.* The effect of some essential oils on the linear growth of the crown rot pathogenic fungus was evaluated *in vitro.* The essential oils, thyme, rose, and lemongrass, at different concentrations were used in this test. The essential oils were added individually to conical flasks containing sterilized PDA medium before solidification to obtain the proposed concentrations of 0.25, 0.5, and 1.0%. A separate PDA flask free of essential oils was used as check (control) treatment. The supplemented media were poured into Petri dishes (9 cm Ø) nearly 20 mL per each. Mycelial discs (5 mm Ø) were taken from the periphery of actively growing PDA cultures of *A. niger*, placed at the centre of the Petri dishes, then quickly sealed with parafilm, and incubated for seven days at 25 ± 1°C. For each treatment, concentrations were tested, and five replicate Petri dish cultures were used. The diameter of the colonies was measured, and reduction in fungal growth was calculated in relation to its growth in check treatment. This test was repeated three times, and the inhibition was calculated as reduction in colony diameter growth compared with the control for each particular tested material.

Antagonistic studies of biocontrol fungi (six isolates of *Trichoderma harzianum*) against *A. niger* were performed in PDA medium in 9 cm diameter Petri dishes following the methods described elsewhere [20]. Inoculated Perti dishes were assigned to a completely randomized design, with five replicates, and incubated at 25 ± 1°C. This test was repeated three times, and the inhibition was calculated as the percentage of reduction in colony diameter growth of pathogenic fungus compared with the control for each particular tested bio-agent.

*2.3. Field Experiments.* Soil treatments, with compost, bioagent *T. harzianum,* and essential oils alone or in combination with compost, were applied under field conditions to evaluate their efficacy against peanut crown rot disease incidence during two successive growing seasons. Field experiments were carried out at the Experimental Research Station of National Research Centre at El-Noubareia region, Behera Governorate, Egypt. This field is well known by the authors as naturally heavily infested with crown rot pathogens during several previous growing seasons which was estimated between 50 and 55%. A field experiment consisted of plots (7 × 6 m); each comprised of 12 rows and 30 holes/row which were conducted in completely randomized block design with five plots as replicates for each particular treatment as well as untreated check treatment. The *T. harzianum* and essential oils, that is, thyme, rose, and lemongrass at concentration of 0.5% alone or in combination with compost, were applied as follows:

(a) single treatments

(1) compost,
(2) *T. harzianum*,
(3) rose 0.5%,
(4) lemongrass 0.5%,
(5) thyme 0.5%;

(b) combined treatments

(6) compost + *T. harzianum*,
(7) compost + *T. harzianum* + rose 0.5%,
(8) compost + *T. harzianum* + lemongrass 0.5%,
(9) compost + *T. harzianum* + thyme 0.5%,
(10) fungicide (Vitavax-Captan at the rate of 3 g/kg seeds as seed dressing),
(11) untreated control.

The inoculum of antagonistic *T. harzianum* was incubated in potato dextrose broth culture on a 170 rpm shaker at 28°C for 5 days. The growth suspensions of *T. harzianum* were adjusted to $10^6$ cfu/mL. The antagonistic bio-agent was mixed thoroughly at the rate of 1.0% (v/w) with compost before soil application. All compost treatments were applied at rate of 20 $m^3$/feddan (4200 $m^2$). Compost and/or bioagent inoculum were incorporated on the top of 20 cm of the soil surface at planting row sites considering relevant treatments. The prepared solution of bio-agents mixture was also incorporated into the same cultivated row site [21]. All applied treatments were applied in completely randomized block design with three replicates (plots) for each particular treatment. The peanut seeds cv. Giza 3 were sown for two successive seasons (2011 and 2012) using the same field design plots of applied treatments. The average percentage of peanut crown rot incidence was recorded until 60 days, of sown date. Determination of peanut yield per $m^2$ was calculated. The average percentage of disease incidence and the obtained yield for the two successive growing seasons were calculated and presented.

Table 1: Effect of different essential oils concentrations on the linear growth of *Aspergillus niger in vitro*.

| Essential oils | Concentrations (%) | *Aspergillums niger* Linear growth (mm) | Reduction % |
|---|---|---|---|
| Thyme | 0.25 | 31.0 c | 65.5 |
| | 0.50 | 00.0 d | 100 |
| | 1.00 | 00.0 d | 100 |
| Rose | 0.25 | 65.0 b | 27.7 |
| | 0.50 | 32.0 c | 64.4 |
| | 1.00 | 31.0 c | 65.5 |
| Lemongrass | 0.25 | 25.0 cd | 72.2 |
| | 0.50 | 00.0 d | 100 |
| | 1.00 | 00.0 d | 100 |
| Control | 00.0 | 90.0 a | — |

Figures with the same letter are not significantly different ($P = 0.05$).

Table 2: Reduction in the growth of *Aspergillus niger* in response to *Trichoderma harzianum in vitro*.

| *Trichoderma harzianum* isolates | Growth area ($mm^2$) | Reduction % |
|---|---|---|
| *T. harzianum* 1 | 12.0 d | 81.1 |
| *T. harzianum* 2 | 18.0 c | 71.7 |
| *T. harzianum* 3 | 21.0 c | 67.0 |
| *T. harzianum* 4 | 19.0 c | 70.0 |
| *T. harzianum* 5 | 31.5 b | 50.5 |
| Control | 63.6 a | — |

Figures with the same letter are not significantly different ($P = 0.05$).

*2.4. Statistical Analysis.* All experiments were set up in a complete randomized design. ANOVA system was used to analyze differences between antagonistic inhibitor effect and linear growth of pathogenic fungi *in vitro* as well as analyze differences between applied treatments and percentages of disease incidence and obtained yield. A general linear model option of the analysis system SAS [22] was used to perform the ANOVA. MSTAT-C program (V2.1) was used to perform the analysis of variance. Tukey test for multiple comparisons among means was utilized [23].

## 3. Results and Discussion

*3.1. Laboratory Tests.* Evaluation the inhibitor effect of some essential oils and antagonistic bio-agent on the growth of peanut crown rot pathogenic fungus was carried out *in vitro*. The inhibitory effect of thyme, rose, and lemongrass oils against the linear growth of *A. niger* was presented in Table 1. The recorded data indicate that all treatments significantly reduced the linear growth of *A. niger*. The fungal growth was reduced by increasing concentrations of tested essential oils. Lemongrass oil seems to have the most inhibitor effect against the fungal growth followed by thyme and rose oils, respectively. Complete reduction was obtained with thyme and lemongrass at the concentration of 0.5%. Meanwhile, lesser effect was observed with rose oil that caused only 65.5% fungal growth reduction at the concentration of 1.0%.

It is well established that some plants contain compounds able to inhibit the microbial growth [24]. These plant compounds can be of different structures and different mode of action when compared with antimicrobials conventionally used to control the microbial growth and survival [25]. Potential antimicrobial properties of plants had been related to their ability to synthesize, by the secondary metabolism, several chemical compounds of relatively complex structures with antimicrobial activity, including alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes, phenylpropanes, and organic acids [26]. In the present study, the chemical compositions of tested essential oils were (−)-citronellol (34–55%), phenyl ethanol (1.5–3%), geraniol and nerol (30–40%), farnesol (0.2–2%), stearpoten (16–22%) with traces of nonanol, linalool, nonanal, phenylacetaldehyde, citral, carvone, citrine for rose oil (http://www.perfectpotion.com.au/Online-Shop/Products/rose-absolute.aspx), myrcene 0.21%, limonene 7.77%, linalool (traces), citronellal (0.25%), citral (79.1%), geranyl acetate (0.85%), nerol (0.33%), geraniol (1.95%), neral, geranial, borneol for lemongrass oil (http://www.perfectpotion.com.au/Online-Shop/Products/lemongrass.aspx) and a-thujone, a-pinene, camphene, b-pinene, p-cymene, a-terpinene, linalool, borneol, b-caryophyllene, thymol, and carvacrol (http://www.essentialoils.co.za/essential-oils/thyme.htm) for thyme oil.

In this regard, the aesthetic, medicinal, and antimicrobial properties of plant essential oils have been known since ancient times. Numerous studies on the fungicidal and fungistatic activities of essential oils have indicated that many of them have the power to inhibit fungal growth. Thyme oil proved to be extremely effective as a fumigant as well as a contact fungicide against a range of the economically significant fungi *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea,* and *Erysiphe graminis* [27]. These biological events could take place separately or concomitantly culminating with mycelium germination inhibition [28]. Also, reports indicated that essential oils containing carvacrol, eugenol, and thymol (phenolic compounds) had the highest antibacterial performances [29].

The antagonistic effect of *T. harzianum* against the growth area of *A. niger* was presented in Table 2. Results indicate that all *T. harzianum* isolates significantly reduced the growth area of *A. niger*. The highest reduction was obtained by isolate nos. 1, 2, 3, and 4 which reduced the growth area by 81.1, 71.7, 70.0, and 67.0%, respectively. Meanwhile, another isolate (no. 5) showed lower inhibitor effect recorded as 50.5%. Biological control of plant diseases, especially soil-borne plant pathogens, has been the subject of much research in the last two decades. Therefore, biological control of plant pathogens is becoming an important component of plant disease management practices. *Trichoderma harzianum* is effective bio-control agent for a number of soil-borne plant pathogens [9, 10]. In present study, results indicate that *T. harzianum* isolates significantly reduced the growth area of pathogenic fungus under *in vitro* conditions. Such results

Table 3: Effect of combined treatments between biocompost and essential oils on crown rot disease incidence (%) of peanut plants under field conditions.

| Treatments | Season, 2011 | | Season, 2012 | |
|---|---|---|---|---|
| | Preemergence | Postemergence | Preemergence | Postemergence |
| Single treatments | | | | |
| *T. harzianum* | 12.0 cd | 14.0 de | 10.0 e | 12.0 f |
| Compost | 15.0 c | 18.0 d | 12.0 de | 19.0 d |
| Thyme | 16.0 c | 22.0 c | 14.0 cd | 22.0 c |
| Rose | 24.0 b | 32.0 b | 23.0 b | 33.0 b |
| Lemongrass | 16.0 c | 23.0 c | 16.0 c | 24.0 c |
| Combined treatments | | | | |
| Compost + *T. harzianum* | 10.0 d | 13.0 e | 12.0 de | 12.0 f |
| Compost + *T. harzianum* + thyme | 7.0 e | 10.0 f | 8.0 f | 7.0 g |
| Compost + *T. harzianum* + rose | 12.0 cd | 16.0 de | 12.0 de | 15.0 e |
| Compost + *T. harzianum* + lemongrass | 6.0 e | 9.0 f | 7.0 f | 8.0 g |
| Fungicide (Vitavax-Captan) | 1.0 d | 10.0 f | 12.0 de | 7.5 g |
| Control | 32.0 a | 55.0 a | 29.0 a | 49.0 a |

Figures with the same letter are not significantly different ($P = 0.05$).

concerning the inhibitory effect of various fungal antagonists on soil-borne plant pathogens were reported previously by many investigators [30, 31].

*3.2. Field Experiments.* Since no alternative to chemical control alone is as consistently effective as fungicides in reducing plant diseases, promising alternatives of biological control with natural compost and plant essential oils treatments were tested to develop a satisfactory control strategy of crown rot disease of peanut. In this regard, certain strategies, such as adding calcium salts, carbohydrates, amino acids, and other nitrogen compounds to bio-control treatments in order to enhance bio-control activity of antagonists against fungal pathogens, were suggested [32, 33]. In the present study, the efficacy of certain essential oils, compost, and the bio-agent *T. harzianum* introduced to the soil, against the incidence of crown rot diseases of peanut, was evaluated under field conditions during two growing seasons (2011 and 2012). Data in Table 3 indicate that all treatments significantly reduced the peanut crown rot disease incidence throughout the two growing seasons. Data also indicate that the combined treatments showed more efficiency for controlling disease incidence than single treatment. It was also observed that the disease incidence at the second growing season was lesser than the first season. The highest reduction was obtained with combined treatments (compost + *T. harzianum* + thyme and compost + *T. harzianum* + lemongrass) which reduced the disease incidence at both pre-, and post-emergence growth stages, respectively. The crown rot incidence at these treatments was recorded as 7.0, 8.0%; 10.0, 7.0% and 6.0, 7.0%; 9.0, 8.0% compared with 32.0, 29.0%; 55, 49% in control treatment, in respective order. Moderate effect was obtained with compost + *T. harzianum* and compost + *T. harzianum* + rose. Meanwhile, single treatments had lesser effect on disease incidence compared with the combined one. Data also showed that the fungicide treatment as seed dressing gave significant disease reduction at pre-emergence stage compared with untreated control. Similar results were also reported by several investigators [2, 34, 35]. They recorded that seed treatments of peanut with Vitavax-Captan at 3 g/kg seed and spraying the growing plants with Benlate at a rate of 2.5 g/L caused a significant reduction in crown rot incidence of groundnut.

The obtained results in the present study are confirmed with previous recorded reports. In this concern, it was reported that biological control agents have achieved success under field conditions. Among the hundreds of organisms identified as potential biological disease control agents, only few have resulted in proving commercially acceptable controls of these diseases [36]. Also, it is evident that antagonistic bio-agent can affect plant resistance to a pathogen either by inducing the basal level of defense reactions immediately after treatment or by enhancing a capacity for rapid and effective activation of cellular defense responses [37]. These reports could confirm the obtained results in the present study. Furthermore, several proposed nonfungicidal approaches, including the use of biological control with antagonistic microorganisms, heat treatment, induction of resistance, natural fungicides and plant extracts, and essential oils, have been extensively studied. Unfortunately, none of them, when used alone, can provide satisfactory levels of disease control when compared with synthetic fungicides [38–41]. Thus, an integrated disease control strategy has been investigated in the present study which is expected to provide high efficacy to bio-control agent, essential oil, and compost in combination. Also, data obtained in the present study (Tables 3 and 4) showed effectiveness of applied compost either alone or in combination with bio-agent and/or essential oils against disease incidence and yield increase as well. Several studies were conducted with the use of compost as control measure against soil-borne diseases. In this respect, the incidence of several soil-borne plant pathogens has also been reduced by using composts made of different raw materials [14–18]. Since [42] first suggested compost could be used as a peat substitute to control root pathogens, bio-control research has increasingly

Table 4: Effect of combined treatments between bio-compost and essential oils on peanut yield under field conditions.

| Treatments | Season, 2011 | | Season, 2012 | |
|---|---|---|---|---|
| | Yield (kg/$m^2$) | Increase % | Yield (kg/$m^2$) | Increase % |
| Single treatments | | | | |
| *T. harzianum* | 5.0 c | 25.0 | 5.4 c | 28.6 |
| Compost | 5.2 c | 30.0 | 5.5 c | 31.0 |
| Thyme oil | 4.5 d | 12.5 | 4.8 d | 14.3 |
| Rose oil | 4.4 d | 10.0 | 4.7 d | 11.9 |
| Lemongrass oil | 4.6 d | 15.0 | 5.0 d | 19.0 |
| Combined treatments | | | | |
| Compost + *T. harzianum* | 6.5 b | 37.5 | 7.0 b | 66.7 |
| Compost + *T. harzianum* + thyme | 7.0 a | 75.0 | 7.6 a | 81.0 |
| Compost + *T. harzianum* + rose | 6.5 b | 37.5 | 7.0 b | 66.7 |
| Compost + *T. harzianum* + lemongrass | 7.2 a | 80.0 | 7.8 a | 85.7 |
| Fungicide | 6.5 b | 37.5 | 7.0 b | 66.7 |
| Control | 4.0 | — | 4.2 | — |

Figures with the same letter are not significantly different ($P = 0.05$).

focused on developing the right combination of composts and antagonistic microbes. *Trichoderma* in combination with composts from agricultural wastes, for example, was used to suppress *Rhizoctonia solani* in cucumber seedlings [19], and *Trichoderma* sp. and sewage sludge compost were used to suppress Fusarium wilt of tomato [17]. Currently, it is believed that a combination of antagonistic microbes with mature compost may be more efficient in inhibiting disease than using single antagonistic microbial strains or compost alone [8, 17, 19]. Utilization of composts to minimize organic waste pollution and to reduce the addition of chemical fertilizers and fungicides in crop production is a promising strategy for both the present and the future. Furthermore, the used essential oils, thyme, rose, and lemongrass, are reported to contain many volatile compounds [43], so it seems that the antifungal effects are the result of compounds acting synergistically. This means that the individual components by themselves are not sufficiently effective. Similar results were also reported by many researchers indicating the efficacy of essential oils as antifungal inhibitors for a large number of soil-borne pathogens [44–46]. The mode by which microorganisms are inhibited by essential oils and their chemical compounds seems to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents [47]. Many authors emphasized that the antimicrobial effect of essential oil constituents has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane. Effect of specific ions due to their addition had a great effect on intracellular ATP content, and overall activity of microbial cells, including turgor pressure control, solutes transport, and metabolism regulation [48].

The obtained yield in response to applied treatments of *T. harzianum* and essential oils alone or in combination with compost was determined. Data in Table 4 indicate that all treatments significantly increased the peanut yield throughout the two growing seasons compared with untreated control. The highest increase was recorded at combined treatments (compost + *T. harzianum* + thyme and compost + *T. harzianum* + lemongrass) which increase the yield more than 75.0, 80.0% and 81.0, 85.7% at the two growing seasons 2011 and 2012, respectively. Moderate yield increase was obtained with (compost + *T. harzianum* and compost + *T. harzianum* + rose) as well as fungicide treatments. They cause an increase of produced yield estimated as 37.5% and 66.7% at the two seasons, respectively. Meanwhile, other single treatments showed lesser effect as well as fungicide treatment. Single treatment of compost causes an increase in yield estimated as 30.0, 31.0% followed by 25.0, 28.6%; 15.0, 19.0%, 12.5, 14.3%, and 10.0, 11.9% at applied treatments, *T. harzianum*, lemongrass oil, thyme oil, and rose oil, in respective order at the two growing seasons. In this concern, it was reported that reduction of disease incidence with fungicides treatments was followed by an increase in fresh and dry weight, shoot and root length, number of branches, number of leaves, number of nodules, and the yield [2, 35]. Moreover, soil amendment with agricultural wastes alone or in combination with bio-control agents was recommended for controlling soil borne pathogens and increasing the yield of many crops. Sugarcane residues (bagasse) degraded by *Trichoderma* spp. was used as soil amendment to improve growth and yield of rice and pea [49].

## 4. Conclusion

Biological control of plant pathogens is becoming an important component of plant disease management practices. In the present study, the fungal antagonists evaluated demonstrated an inhibitory effect against crown rot pathogen under laboratory and field conditions. The major problem of applying antagonists to soil is their inability to become established in the ecosystem and to overcome the resistance of soil microbiota to the introduction of new microorganisms [50]. The results obtained in the present work indicate that the applied formula containing combination between bio-agent, essential oil, and compost enhanced the efficacy of crown rot incidence of peanut during the two successive growing seasons better than each individual component.

## Conflict of Interests

The authors declare that they do not have a direct financial relation with the two mentioned companies "El-Nile Company, Egypt, and Chemical Industrial Development Company (CID), Egypt," that might lead to a conflict of interests.

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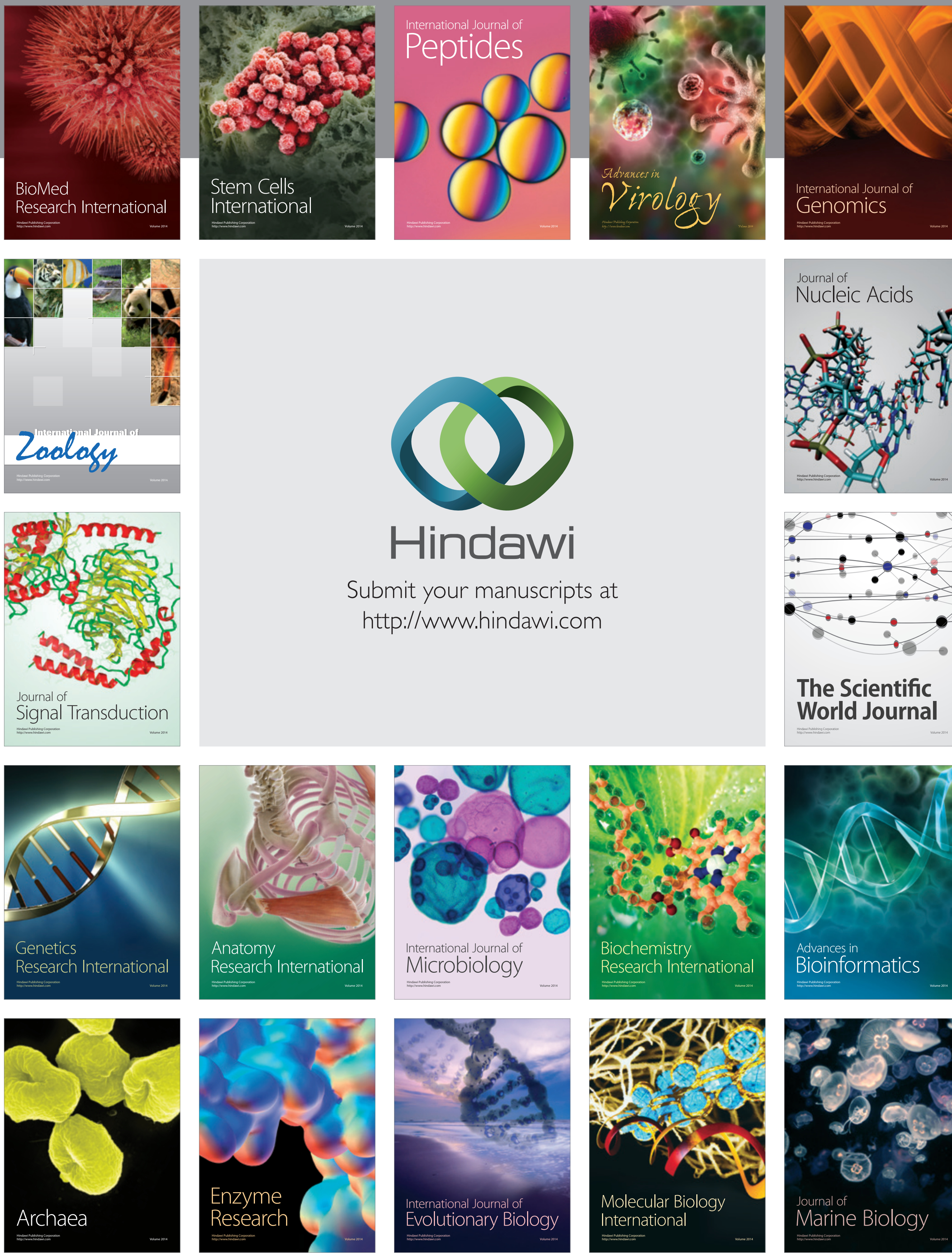

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