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Original article

Comparison of the effectiveness of selected essential oils with mineral oil and spinosad on *Dermanyssus gallinae*

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Abstract

It seems that essential oils can be a good ingredient in effective preparations against *Dermanyssus gallinae*. Dermanyssus affects animal health leading to financial losses and bird welfare issues. Collected mites were treated with various essential oils in four (20, 50, 80 and 100%) concentrations at a dose of 0.28 mg/cm^2. The direct toxicity of the essential oils, mineral oil and spinosad to *D. gallinae* was tested in the laboratory. Eucalyptus oil was the most toxic essential oil in all concentrations to *D. gallinae* (87.6 - 97.6% mortality at all four concentrations), while geranium, pine and rosemary oils showed mortality rates of 14.2 - 68.2%. High mortality after 48 hours of contact was also recorded for the oil of cloves at 80% dilution (85.1% mortality), lavender 100% (94.2% mortality). Similarly, the thyme essential oil produced 83.5 - 93.2% mortality in three concentrations: 50, 80 and 100%. The mineral oil was the least effective oil against mites. Spinosad showed high effectiveness against *D. gallinae*.

Key words: Dermanyssus, mite, essential oil, mineral oil, spinosad

Introduction

Dermanyssus gallinae (De Geer 1778) (Dermanyssidae) is a periodic ectoparasite, often attacking poultry, as well as other species of birds, mammals and even humans. Commonly occuring Dermanyssus infections are observed in all EU countries, as well as in the USA, China and Japan (Chauve 1998, Sparagano et al. 2009), Tunisia (Gharbi et al. 2013), Iran (Rezaei et al. 2016). Research by Kowalski and Sokół (2009) showed that mites feeding on hens causes somatic and psychogenic stress and lowers the humoral immunity of hens (Kowalski and Sokół 2009). By puncturing the skin and taking blood, mites cause anxiety and irritability in birds, as well as itching and sleep disturbance. Chickens peck, scratch, and self-mutilation and cannibalism

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occurs (Boberek and Gaweł 2017). In the case of intensive infestation, anemia, bleeding, and very poor condition of the birds are noted, which eventually lead to death (Wójcik et al. 2000, Cosoroaba 2001). The result is a pathophysiological mechanism of reduced egg production and higher mortality in laying hens (Kowalski and Sokół 2009). In addition, in poultry houses infested with mites, increased feed consumption is observed (Chauve 1998, Mul et al. 2009). Due to the possibility of transmission of many pathogens, mites can affect the spread of epidemiological threats among poultry (Hoffmann 1987, Sparagano et al. 2014, Sommer et al. 2016, Raele et al. 2018). This means a significant impact on the production and welfare of laying hens leading to enormous production losses. The costs associated with control and production losses are estimated at EUR 231 million per annum for the egg industry in the EU (van Emous 2017). Worldwide control of *D. gallinae* is mainly based on the use of synthetic acaricides, such as organic phosphates, organochlorines, pyrethrin, pyrethroids, carbamates, amitraz, and endectocides (Sparagano et al. 2014), and vary depending on the hen housing system. Restrictions in the use of carbamates and organophosphorus and the increasing genetic resistance of mites to approved acaricides make combating this invasion a great challenge (Beugnet et al. 1997, Fiddes et al. 2005, Thind and Ford 2007, Marangi et al. 2009, Circella et al. 2011, Piskorski et al. 2011). Among the other ways of controlling mites are new synthetic acaricides belonging to the phoxime group (Meyer-Kuhling et al. 2007) as well as preparations containing diatomaceous earth, kaolin and silica (Maurer et al. 2009). The use of the Thermo-Kill method also shows promise. Another possible solution is the introduction of a shortened light cycle in the poultry house (Sokół et al. 2008), integrated pest management (IPM) (Axtel 1999) or the use of special traps (Sokół and Romaniuk 2006). Spinosad seems to be a fairly effective active biopesticide, its effectiveness is 95% - 97% (George et al. 2010). An additional advantage of using spinosad, demonstrated in in vivo studies, was the lack of effect of the preparation on the body weight of chickens or the parameters of egg production (number and weight) (George et al. 2010). The battle against *D. gallinae* is also made difficult by factors such as: concealment in hard-to-reach places, long survival rate without feeding, and a very short life cycle. In addition, the great ease of host change and the wide range of *D. gallinae* hosts in comparison to other species of the genus Dermanyssus, means that the parasite stays in the environment (Roy et al. 2009). A separate issue is the residue of the active substances used in eggs and meat, which may pose a threat to human health (Cernea et al. 2006, Kim et al. 2007), contamination of the environment with chemical plant protection products and toxicity for non-target organisms (Isman 2008). Consequently, there is an urgent need to look for alternative methods of limiting these ectoparasites. Many plant species produce toxic secondary metabolites that limit attacks of herbivorous insects and can thus limit the spread of insects on new hosts (Piskorski et al. 2011). So far, studies have shown that numerous essential oils and plant extracts have a high acaricidal potential (Kim et al. 2004, Kim et al. 2007, George et al. 2008, Maurer et al. 2009, George et al. 2009a, Magdas et al. 2010, Martinez-Velazques et al. 2011, Nechita et al. 2015, Immediato et al. 2016, Rajabpour et al. 2018, Lee et al. 2019, Tabari et al. 2020, Bordin et al. 2021). Guimarães and Tucci (1992) and Maurer et al. (2009) showed that refined mineral oil can be used for the control of mite infestations in poultry (Guimarães and Tucci 1992, Maurer et al. 2009).

The aim of the present study was to compare the acaricidal effectiveness against *D. galliane* of selected essential oils, with mineral oil and spinosad.

Materials and Methods

Mites

Dermanussus gallinae colonies were collected from battery cage farms of laying hens from south-eastern Poland. The farm was naturally infected by parasites and no acaricides had been used for two months prior to collection. The collected mites were transferred to tightly closed plastic containers using a metal scapula (150 ml) with about 1-3 g of parasites in each container. The mites were stored at room temperature until tested, to acclimatise the population to the laboratory conditions. The container was aerated daily by unscrewing the lid (2-3 times). Identification of the mites was provided according to the key of Di Palma et al. (2012) (Di Palma et al. 2012). The mites were used in tests within 4 days of collection. Only adult female mites that had fed were used for the experiment, only those that had actively gathered on the lid of the container.

Plant essential oils, mineral oil and spinosad

Eight essential oils (Table 1) used in this study were produced by Etja (Elbląg, Poland). All oils were extracted by a steam distillation process, derived from eucalyptus, lavender, thyme, rosemary, pine, clove, geranium, citronella, were tested for the acaricidal activity.

For the toxicity bioassay, a 20% 50%, 80%, 100% dilution of each essential oil in distilled water was made with 0.28 mg/cm^2 of the oil.

Table 1. List of essential oils tested for acaricidial activity.

Common name	Plant species	Plant part used	Place of origin of the plant
Clove Bud	*Eugenia caryophyllus*	flower buds	Madagascar
Lavender	*Lavandula angustifolia*	flower heads	France
Eucaliptus	*Eucalyptus globulus*	tree twigs and leaves	Australia
Thyme	*Thymus vulgaris*	thyme herb	Italy
Rosemary	*Rosmarinus officinalis*	rosemary bush	France
Pine	*Pinus sylvestris*	pine needles	Russia
Geranium	*Pelargonium graveolens*	flowers	France
Citronella	*Cymbopogon winterianus citronella*	stems and leaves	India

Spinosad produced by Elanco (Poland) with a concentration of 30 ml/ 3.5 l water was tested, at a rate of 0.28 mg/cm^2.

Mineral oil produced by Sigma-Aldrich (Darmstadt, Germany) was used at a rate 0.28 mg/cm^2 per veneer disc.

Evaluation of essential oils efficacy

Toxic properties of essential oils against *D. gallinae* were tested using a modification of the Zdybel at al. (2011) method. Efficacy of the acaricides was carried out on plexiglas plates (patent numer P-376067) with a veneer disc in order to imitate a rough surface, reflecting conditions in a henhouse. The veneer disc (diameter 90 mm) was cemented with aquarium silicone to the central field of the plate. The grooves of each plate were filled with edible oil. The plates were situated in a plastic chamber (22 cm x 22 cm). All the solutions were spread on the surface of the discs and allowed to dry for 15 min at room temperature. For the control group, discs were moistened only with water. Mites were placed on the disc (100 individuals). The mites were then examined after 48 h of exposure to the essential oils to determine mite mortality using a binocular microscope. Mites were considered dead if no movement was visible even after a gentle touch with a needle. For each plate containing a disc moistened with a solution of essential oil, mineral oil and spinosad, the mortality rate of mites was calculated, with the correction taking the mortality in the control group into consideration (Abbott correction). An average constituted the final count from four repetitions.

Statistical analysis

The percentage mortality of mites (dead mites/total mites × 100) with Standard Errors (±SE) and Confidence Intervals (95% CI) under each treatment was calculated. The correction for the oil's effectiveness (%) was made using Abbott's test (dead mites in treatment – dead mites in control/100 – dead mites in control × 100). Percentage data were first arcsine transformed and then analysed using ANOVA tests. One-way analysis of variance was used to make a comparison of mortality between the tested groups' treatment at different exposure times with Tukey's RIR as the post-hoc test, and repeated measures analysis of variance was used to compare different treatments on three consecutive days. A value of $p<0.05$ was considered statistically significant. Statistical analysis was performed with TIBCO Software Inc. (2017). Statistica (data analysis software system), version 13. http://statistica.io.

Results

Detailed results of our experiment are shown in Fig. 1 and Fig. 2. The comparison of 100% concentration of 8 essential oils against *D. gallinae* to mineral oil and spinosad is shown in Fig.1, while the acaricidal effect of the 8 essential oils, at different oil concentrations, is shown in Fig. 2.

The acaricidal effect of 20%, 50%, 80% and 100% dilutions of eight essential oils at an application rate of 0.28 mg/cm^2, varied depending on the plant species and the dilution used. The Eucalyptus essential oil produced 87.6 - 97.6% mortality at all four concentrations against *D.gallinae*. Similarly, the thyme essential oil produced 83.5 - 93.2 - 91.2% mortality in three concentrations, respectively 50-100 and 80% although activity decreased to 20.6% mortality when the 20% dilution was reached. High effectiveness after 48 hours of contact, was also recorded for the Oil of Cloves 80% dilution (85.1% mortality), Lavender 100% (94.2% mortality). A low efficacy was recorded for the oils of: Clove 20% and 50% dilution and Lavender 20% dilution. The following oils also showed low effectiveness in all concentrations: Rosemary, Pine, Geranium and Citronella. The mortality rate for Spinosad was 95.6%, and for Mineral Oil 12.5%.

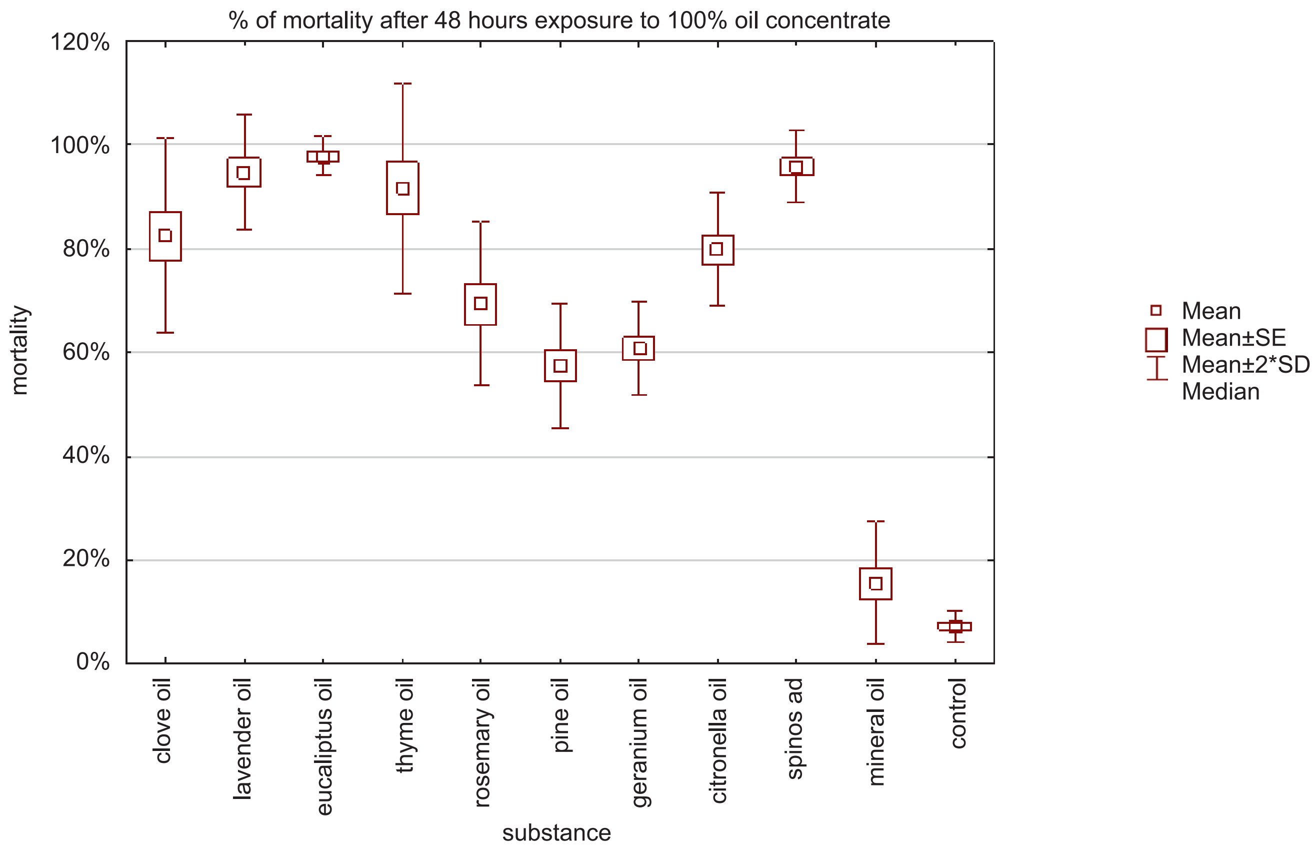

Fig. 1. Comparison of 100% concentration of 8 essential oils against Dermanyssus gallinae to mineral oil and spinosad.

Discussion

Plants defend themselves against attack by arthropods by producing numerous chemical compounds. Plant oils and extracts have antiseptic, anti-inflammatory, antifungal and antibacterial properties (Prabuseenivasan et al. 2006). Many essential oils are known to be biocidal against various arthropods, but most importantly have little or no harmful effects on non-target organisms (Isman 1999). Due to their promising biocidal and repellent properties, they can be perfect natural alternatives to synthetic acaricides. In the present study we researched the acaricidal activity of eight essential oils against *D. gallinae* and compared the acaricidal effect of essential oil to spinosad and mineral oil. We wanted to exclude the risk of the coating and clogging of spiracles that occurs after oil spraying. When spraying in a poultry house, it is not possible to reach all mites and not all of them will be sprayed equally. Therefore, when performing our research, we took into account the fumigation properties of the tested compounds, as well as the contact properties, but the results reflect only those that occur after spraying the surface on which the mites are put. Oils work physically: they cover the bodies of insects or mites with a thin membrane and penetrate capillaries through their spiracles, causing them to clog (resulting in irritation and death by suffocation). They only work by contact, but the insecticidal action is much more effective if the mixture is thoroughly sprayed over the entire surface of the parasites (Ciesielska et al. 2011). Essential oils work through contact action and fumigation action. Some aromatic plants contain volatile compounds (allelochemicals), which are known to possess insecticidal and insect-repellent activities (Jilani et al. 1988, Shaaya et al. 1991). George et al. (2009b) studied the action of Thyme, Manuka and Pennyroyal oil and demonstrated that mites exposed to the gaseous phase of the essential oil both with and without contact with the oil itself, showed a lethal effect. The effect was higher in closed containers than in open containers (George et al. 2009b). Kim et al. (2004) reported the acaricidal activity of some plant extracts and 56 essential oils. In their research on contact bioassays, 100% lethal effectiveness was achieved by the following oils: bay, cade, cinnamon, clove bud, coriander, horseradish, lime, mustard, pennyroyal, pimento berry, spearmint, red thyme and white tyme, all used at a dose of 0.07 mg /cm^2 (Kim et al. 2004). In the fumigation test, however, he showed that closed containers were more effective than open ones. Thus, for clove bud, coriander, horseradish and mustard oils at 0.28 mg/cm^2 the mortality rate was significantly higher than the open container method, which might suggest that the effect of these es-

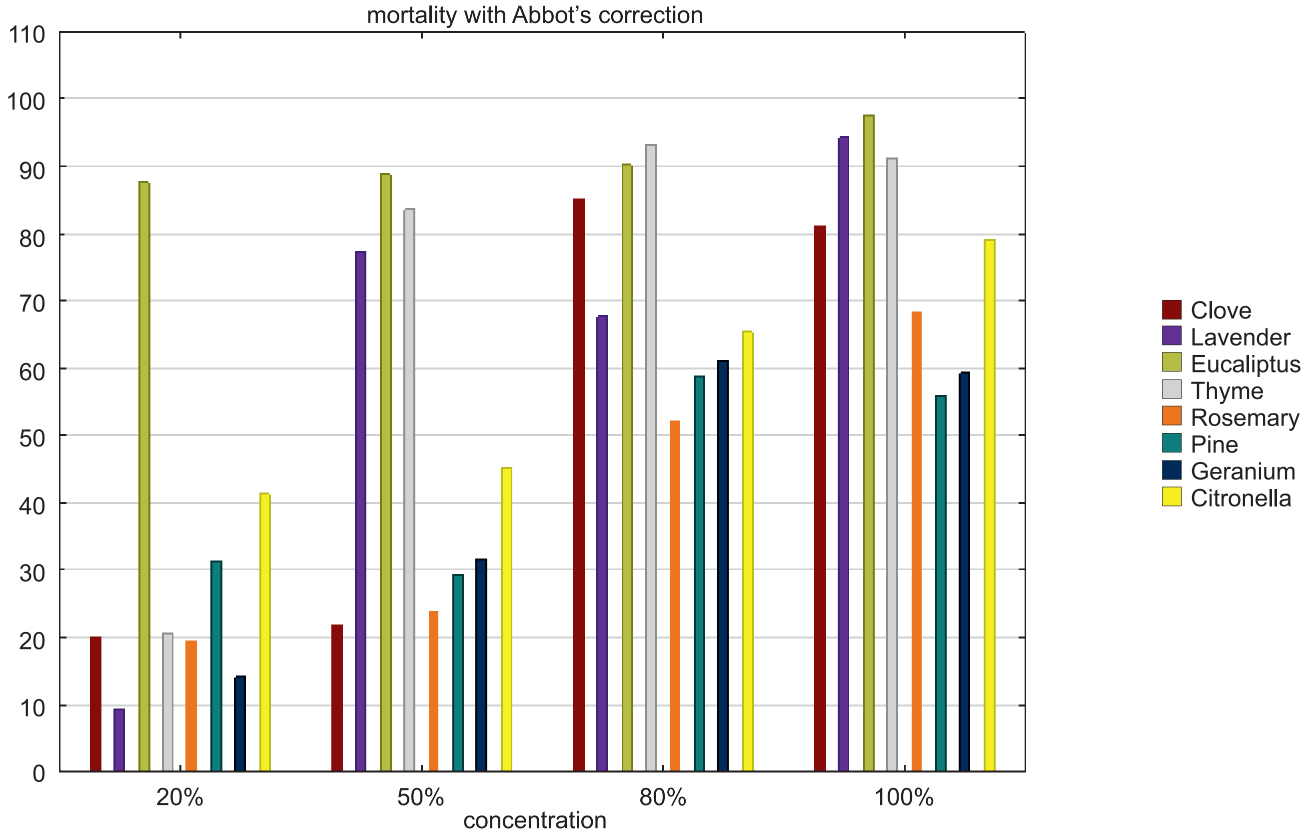

Fig. 2. Acaricidal effect of 8 essential oils at different oil concentrations after Abott's correction.

sential oils can be largely due to action in the vapor phase (Tabari et al. 2017). In our study, few essential oils tested had significant effects on *D. gallinae* under laboratory conditions. The toxicity effect was dose--dependent and varied with plant species. Eucalyptus oil had the best killing effect, having a high level of effectiveness in all four concentrations. Thyme, clove and lavender oils also had high effectiveness of action, but at the higher concentration used. We also noticed that the death of the mites was not immediate. After approximately 8 hours after applying the mites to the plate, using essential oils and Spinosad, the mites clustered together. Then, approximately 12 hours after the start of the experiment, the mites were dead in the case of eucalyptus, thyme, clove and lavender oils. This phenomenon was not noticed in the case of using mineral oil. From the literature data it appears that poultry red mite (PRM) can release pheromones which attract other PRM causing mites to communicate among themselves and cluster together (Entrekin and Oliver 1982, Koenraadt and Dicke 2010, Gay et al. 2020). This phenomenon is being monitored and will be investigated in more detail in subsequent experiments. Some researchers have isolated individual chemical active ingredients contained in oils and study their effects on arthropods (George et al. 2009a, Sparagano et al. 2013, Kim et al. 2016, Tabari et al. 2017, Chen et al. 2019, Radsetoulalova et al. 2020). Studies show that the acaricidal effect of complete oils is more effective than the action of combinations of their individual active components. Miresmailli et al. (2006) claims that this may be the result of the collaboration between the active and inactive ingredients in the oil (Miresmailli et al. 2006). Comparing our research with other researchers, essential oils from the same plant species differ in their effectiveness (Kim et al. 2004, Moreno et al. 2007, Isman 2008, George et al. 2008, Magdas et al. 2010, Nechita et al. 2015). This may be due to the fact that the distribution of individual components in the oil is variable and depends on the geographical origin of the plant (Munoz-Bertomeu et al. 2007, Negahban et al. 2007, Raal et al. 2007), seasonality (Flamini and Cioni 2007), the harvest year, storage conditions (Chalchat et al. 2007), oil extraction method (Chiasson et al. 2001) and parts of the plant (Stešević et al. 2016). Differences in the effectiveness and action of the oils may also result from the degree of mite satiation and their weakening resulting from acaricides previously used in the henhouse. George et al. (2008) suggest that *D. gallinae* is more susceptible to the effects of essential oils after fasting for 3 weeks. Our mites were fed, so we can exclude in this situation the issue of greater susceptibility to the action of essential oils of starving mites, as our mites were tested within 4 days of collection.

Furthemore, the *D. gallinae* colonies were harvested from a farm naturally infested with parasites and no acaricides had been used for two months prior to collection. As mentioned, the mites were applied to the plates previously sprayed with mineral oil, so it is not surprising that in our study the mineral oil did not show an enhanced lethal effect. In contrast, in research by Guinnares and Tucci (1992), mineral oil caused 100% mite mortality just 2 hours after spraying. However, in the study mentioned above, the mites were completely covered with sprayed oils (Guinnares and Tucci 1992). In a study by Maurer et al. (2009), mites came into contact with filter paper disks saturated with petroleum spray oil and diesel oil. The mite mortality after 7 days of treatment was approximately 95% (Maurer et al. 2009). Spinosad, which is a natural product derived from the fermentation of the micro-organism *Saccharopolyspora spinosa*, also exhibited a high toxic effect and we used this as a positive control. Our research showed that its effectiveness was 95.6%. Spinosad has been found to be active against a range of insect pests, especially those in the genera Lepidoptera, Diptera and Thysanoptera, and to a lesser extent the Coleoptera and Orthoptera genera (Thompson et al. 2000). The mode of action of spinosad is characterized by excitation of the nervous system leading to subsequent paralysis and death (Anastas et al. 1999, Thompson et al. 2000). As can be seen in Fig. 1, where differences between means as identified by Tukey's Tests are shown 100% lavender, eucalyptus and thyme essential oil were at a similar level to spinosad. Most of the terpenoids and phenols found in plant essential oils have minimal vertebrate toxicity and have been approved as GRAS (Generally Regarded As Safe) compounds by the US Food and Drug Administration (Kostyukovsky et al. 2002). Considering possible environmental contamination of synthetic pyrethroids and the eventual toxicity to non-target organisms, essential oils against *D. gallinae* are recommended as natural repellents.

In conclusion, the results obtained confirm that selected essential oils are toxic to *D. gallinae* at in vitro conditions, while the mineral oil is non-lethal to mites when exposed to contact. Spinosad showed high effectiveness against *D. gallinae* and its action is comparable to that of eucalyptus oil.

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