Plant extracts as plant strengtheners: effects on cucumber and *Thrips tabaci*

Master thesis

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Abstract

The plant strengthening effect of *Artemisia absinthium* extract, *Tanacetum vulgare* extract and the plant strengthening product “Pflanzen Vital Spray S” (containing both *A. absinthium* and *T. vulgare* extracts), against *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber (*Cucumis sativus*, var. Korinda) was evaluated using leaf disc bioassays and histological characterisation of cucumber-leaf sections. To exclude a direct toxic effect on the pest, the direct contact toxicity of the products was tested on 2nd instars of *T. tabaci.* No significant increase in the 24 and 48 h mortality rate could be observed compared to water or zero treatments. Feeding and ovipositional rate were evaluated using a no choice leaf disc bioassay. No significant differences could be detected between product and water treated plants. Choice leaf disc bioassays were conducted to check for settling and ovipositional preference of *T. tabaci*. Leaves of *A. absinthium* and “Pflanzen Vital Spray S” treated plants were generally less preferred then water treated leaves but no influence on the ovipositional preference could be detected. Leaves from *T. vulgare* treated plants did not result in significant differences in the settling preference but thrips preferred to lay eggs on *T. vulgare* treated leaves over water treated ones. For the histological characterisation, thin leaf sections were produced using Vibratome and Microtome. Vibratome sectioned leaves were checked for differences in the epidermis thickness. *A. absinthium* and *T. vulgare* treated plants had thicker epidermis cells then water treated plants. Leaf cross sections produced by means of Microtome were checked for differences in the thickness of epidermis cell walls. All three treatments had thicker cell walls then the water treated control leaves. Additional staining of callose and lignin exhibited no differences between the treatments. Staining of pectin resulted in a higher coloration within the product-treated leaves compared to the water treated ones.

Key Words: *Artemisia absinthium*, *Tanacetum vulgare*, Onion thrips, Thysanoptera,

plant strengthener

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1. Introduction

In the non-scientific literature on organic horti- and agriculture, a wide variety of herbs are advertised to increase the resistance of crops against pests and phytopathogens. The underlying mode of action is described as a “strengthening of plants or plant tissues” (Berg 2011; Rusch 2010) or a “strengthening of the plant’s defences” (Kreuter 2012). It is even claimed that the epidermal cells are thickened or “protective substances” are embedded in the epidermis (Achting et al. 2004) which should lead to a higher resilience of crops. In order to strengthen plants, authors recommend the preparation of teas or decoctions of different kinds of herbs like comfrey, stinging nettle, horsetail, bracken, common tansy, wormwood, onion or garlic and the application of those on the crops by spraying (Grault 2009; Kreuter 2012; Maier 2005).

When looking into scientific literature, a proof for measurable effects of plant strengthening products against insect pests is lacking. There is research done on plant-strengthening effects against phytopathogens. Fofana and co-authors could verify a protective effect of a plant strengthener called “Milsana”-a product based on extracts of Giant Knotweed (*Reynoutria sachalinensis* L.) - against powdery mildew of cucumber. The underlying mode of action was identified as an induction of the chalcone synthase, which resulted in the accumulation of a defence-related flavonoid after the infection with the fungus. (Fofana et al. 2002; Treutter 2010). Furthermore there is proof for the plant strengthening effect of algae, or substances derived from algae such as Laminarin or oligo carrageenans, against pathogens such as *Botrytis cinerea*, *Plasmopara viticola* or other bacterial, viral or fungal infections (Vera et al. 2012; Bulgari et al. 2015). Additionally research is done on the effect of plant strengtheners on the attractiveness of crops to beneficial insects (Sobhy et al. 2014) and on the effect of substances such as Acibenzolar–S-Methyl in inducing systemic resistance against pathogens in plants (Lorenz et al. 2015; Tripathi and Pappu 2015). But research on herb based plant strengthening preparations against insect pests is still lacking.

A highly harmful pest insect is the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). This tiny insect is an important polyphagous, sap-feeding pest worldwide with a broad variety of vegetable and ornamental host plants (Hill 1994; Murai 2000; Emden 2013). *T. tabaci* causes direct damage to crops by damaging the surface of leaves or other plant parts with its piercing and sucking mouthparts, causing a silvery appearance (Kirk 1997; Pedigo 2002). Infestation of crops can lead to high yield losses and – especially in ornamental plants – to cosmetic damages (Kirk 1997). Additionally, *T. tabaci* can cause indirect damage by acting as a vector for tomato spottet wilt virus (TSWV) and Iris yellow spot virus (Mound 1996; Jones 2005; Diaz-Montano et al. 2010; Macharia et al. 2015). By decreasing yield, market value and storage duration of crops, the economic impact of this pest is tremendous and the reason why different means of plant protection measures are applied to protect crops from thrips feeding (Kirk 1997; Lewis 1997 c; Liu and Sparks 2003; Nault and Shelton 2010).

Because of its distinct habit of crawling into very small spaces and hiding in narrow crevices, which is called thigmotactic behaviour (Mallmann 1959 and 1964; Mound 1996; Childers 1997) chemical control of *T. tabaci* is challenging. Additionally its ability to develop resistances to many active ingredients (Lewis 1997 a; Martin et al. 2003; Allen et al. 2005; Foster et al. 2010) and the rising consciousness for the environmental and health impacts of synthetic pesticides (Jeyaratnam 1990; Alavanja 2004; Pretty and Hine 2005) are driving forces for the search of new alternatives to synthetic pesticides.

One alternative - which would be easy to combine with other non-chemical strategies of plant protection - could possibly be the application of plant strengtheners on crops. *Artemisia absinthium* L. (Asteraceae) a yellow flowering perennial plant native to the drier regions of Europe and Asia (Czygan et al. 1994; Abad et al. 2012) and *Tanacetum vulgare* L. (Asteraceae) also native to Europe and parts of Asia, are medicinal plants which are used since centuries for a variety of pharmaceutical purposes (Chevalier 2001). *A. absinthium* is used to stimulate the appetite, for gastrointestinal complaints and for its antiparasitic effects (Czygan et al. 1994;

Abad et al. 2012). Further, *A. absinthium* essential oils are used as insecticides or insect repellents (Chiasson et al. 2001; Ertürk 2006; Mediouni 2014). *T. vulgare* was used to kill worms in the human body and flees or lice on the body, but because of its toxicity it is not used anymore in human medicine (Ennet et al. 1990; Chevalier 2001). Additionally *T. vulgare* was used by Native Americans as an insect repellent and extracts of *T. vulgare* reportedly have antifeedant effects against some insect pests (Chiasson et al. 2001). Today plant strengthening products based on *A. absinthium* and *T. vulgare* are available on the market and are advertised to increase the resistance of crops against insect pests. But whether repeated applications of plant extracts really affect growth and/or cell wall characteristics, thus "strengthen" the treated crop, and as a consequence affect feeding and oviposition of sap-feeding insects such as *T. tabaci* has not been investigated to date. This is the reason why this research was carried out. The effect of products, based on extracts of *A. absinthium* and *T. vulgare* on the settling and oviposition preference as well as the feeding and ovipostion rate of *T. tabaci* on cucumber was studied. Additionally the influence of those products on the plant's leaf tissue was evaluated by checking thin leaf sections for histological changes. The thickness of the leaf's upper epidermis was measured as well as the thickness of the epidermal cell walls. Additionally leaf sections were stained for pectin, lignin and callose to look for any visible differences between the treatments and the control.

The objectives of this master thesis are to answer the following questions:

1.) Do *A. absinthium*, *T. vulgare* or "Pflanzen vital spray S" increase the mortality rate of *T. tabaci* 2nd instars 24 and 48 hours after treatment?
2.) Do *A. absinthium*, *T. vulgare* or "Pflanzen vital spray S" have a repellent/deterrent effect on the settling and ovipositional preference of groups of *T. tabaci* adults on cucumber leaf discs punched from differently treated cucumber plants or untreated plants?
3.) Do *A. absinthium*, *T. vulgare* or "Pflanzen vital spray S" have a deterrent effect on the feeding and ovipositional rate of single *T. tabaci* adult females on leaf discs from treated or untreated cucumber plants?

4.) Do A*. absinthium*, *T. vulgare* or “Pflanzen vital spray S” have an influence on the histological structure of cucumber leaves? Do A*. absinthium*, *T. vulgare* or “Pflanzen vital spray S” increase the amount of pectin, lignin or callose in treated leaves?

2. Plant strengthener - definitions

When looking into the literature, the terminology of substances that are used to increase the resilience of plants, is often not precise and there are many terms meaning the same: plant strengtheners, plant conditioners, biostimulants, plant activators and elicitors. The German federal office of consumer protection and food security defines plant strengtheners as “substances and mixtures including microorganisms which either are exclusively intended to maintain plant health in general as long as they are not plant protection products according to Article 2 (1) of Regulation (EC-European Commission) No 1107/2009 or are intended to protect plants against non-parasitic impairments” (www.bvl.bund.de). In the Austrian legislation plant strengtheners are described as plant conditioners (Pflanzenhilfsmittel) and defined as: “agents without any significant nutritional value that are meant to effect plants, increase the resilience of plants or influence the processing of organic matter.” (Düngemittelgesetz 1994, English translation by the author). All products which are classified as “plant strengtheners” in Germany can be used as “plant conditioners” in Austria (BMLFUW, 2013).

Whereas “Plant biostimulants, or agricultural biostimulants, include diverse substances and microorganisms that enhance plant growth” (Calvo et al. 2014), “Plant activators are chemicals that induce plant defence to a broad spectrum of pathogens” (Sun et al. 2015). Elicitors are referred to as substances that can elicit “systemic acquired resistance” or “induced systemic

resistance" in plants, thereby increasing their defensive capacity against pathogens and pests (Goodman and Vallad 2004).

All products tested in this research are classified as plant strengtheners by the federal office of consumer protection and food security of Germany and listed in their list of plant strengthening substances (BVL 2015), which means they are also allowed to be used in Austria as plant conditioners.

3. Material and methods

3.1 Test plant and application procedure

Cucumber (*Cucumis sativus*, var. Korinda) plants were sown in peat based substrate (Profisubstrat ED 63 T - Einheitserde, Germany) in pots and grown inside a plant growing room at 25 °C, 33 % relative air humidity and a photoperiod of 16:8 (light/darkness). Five treatments were established: Plants treated with one of the three test products (see 3.2 Test products), plants treated with water (water-treatment) and plants not treated at all (zero-treatment). For each treatment cucumber plants were sprayed with the plant strengthening products and afterwards kept away from the lamps as long as they had a wet leaf surface to avoid burning-damage on the leaves. Plants were sprayed with the products using uniform spray bottles that enabled an evenly distribution of the products on the leaf surface.

The first spraying treatment took place 14 days after sowing, the second 21 days and the third 28 days after sowing. For the first treatment small spray bottles were used and about 0.8 ml product and respectively water was applied per plant. For the second treatment about 20.5 ml and for the third treatment about 27 ml were applied per plant using bigger spray bottles. On every treatment all leaves of each individual cucumber plant were sprayed on the upper leaf

surface. Thirty days sowing leaves from all ages were randomly harvested. For usage in the bioassays leaf discs punched from fresh leaves were used. For the histological characterization, leaves were conserved in 30 % ethanol.

3.2 Test Products

The plant strengthening effect of three products was tested: *A. absinthium (*commonly named wormwood) extract (F. Schacht GmbH & Co. KG, Germany), *T. vulgare* (commonly named common tansy) extract (F. Schacht GmbH & Co. KG, Germany), and "Pflanzen Vital Spray S" (Florissa Handels- und Produktions-GmbH, Germany), a ready-to-use product, available as a spray bottle containing 500 ml liquid with 995 g/L extracts of both *A. absinthium* and *T. vulgare*. For reasons of space this product is simply termed "Florissa" in the figures. *A. absinthium* contains 0.15-0.4 % bitter substances with absinthin as its main component and 0.2-1.5 % essential oil with terpenes like β- thujone, camphor and 1,8 cineol as its most abundant components (Czygan et al. 1994; Bachrouch et al. 2015). *T. vulgare* flowers contain up to 1.5 % essential oil and also flavonoids and bitter substances like Tanacetin (Ennet and Reuther 1990). Wormwood and Common tansy extracts were formulated as teas. Following the instructions on the package the tea was prepared from 5 g herb extract with 1L boiling water. Afterwards the tea was let brewing for 15 minutes and was then filtered through a filter paper (diameter 90mm). After approximately half an hour the tea was cool enough to be sprayed on the test plants. Fresh tea was prepared for every spraying treatment. "Pflanzen Vital Spray S" was filled in the same spray bottles as the teas and then applied to the cucumber plants.

3.3. Test insect: Thrips tabaci

T. tabaci commonly named onion thrips, is a world-wide distributed pest that can colonize a wide variety of agricultural and horticultural crops between sea level and 2000 meters above sea level (http://web.entomology.cornell.edu/shelton/veg-insects-global/english/thrips.html 26.8.2015).

3.3.1. Taxonomic classification (Mound and Kibby 1998)

Class: Insecta

Subclass: Pterygota

Order: Thysanoptera

Suborder: Therebrantia

Family: Thripidae

Subfamily: Thripinae

Genus: Thrips

Species: *Thrips tabaci* Lindeman

3.3.2. Morphology

T. tabaci is a tiny, usually only about 1 mm long insect (Emden 2013). Its colour ranges from pale yellow to dark brown (Moritz 2006). The dorsoventrally flattened body of adult *T. tabaci* individuals can be differentiated into head, thorax and an abdomen consisting of 11 segments (Moritz 1997). The female abdomen has a conical apex, whereas it is rounded in males (Hill 1994). The four wings are membranous, slender and characteristically fringed with cilia (Lewis 1997 b).

The head of *T. tabaci* is broader than it is long (Moritz 2006). It bears mouthparts which are described as “piercing-sucking”. By using its single left mandible *T. tabaci* pierces the plant tissue and sucks up the juice of individual leaf cells with a sucking tube that is formed via a combination of their two maxillary stylets (Mound 1971; Kirk 1996, 1997; Moritz 1997). Antennae of *T. tabaci* are inserted into a socket on the head capsule. They consist of 7 segments and bear the sense organs of the insect (Moritz 1997, 2006).

Females have a large, downward curved ovipositor that is used to pierce the plant tissue and deposit eggs (Terry 1997; Alford 1999).

3.3.3. Reproduction and life cycle

Populations of *T. tabaci* can reproduce in two modes: sexually (arrhenotokous) or asexually (thelytokous) (Moritz 1997; Jenser and Szenasi 2004). In the asexual reproduction via parthenogenesis females develop from unfertilised eggs (Li et al. 2014). The two modes of reproduction can coexist in one population (Kobayashi et al. 2013; Li et al. 2015).

T. tabaci has 6 development stages: egg, two instars, prepupa, pupa and imago (Van Rijin et al. 1995; Lewis 1997 c). While imago and the two instars are actively feeding and thereby causing damage to crops, prepupa and pupa hide in protected places like soil or plant cavities and are relatively inactive and non-feeding (Kirk 1996; Deligeorgidis and Ipsilandis 2004). The two wingless larva stages, which look similar in shape to the adults are whitish to yellow and there is a moult between the two (Kirk 1996).

3.3.4. Damage and symptoms of infestation

By scarring the surface of leaves, stems and fruits with their piercing sucking mouthparts, feeding of *T. tabaci* characteristically leads to a so called silvery appearance (Childers 1997; Kirk 1997; Emden 2013). This damage symptom can be explained by the entering of air into sucked out parenchyma cells which then appear as silvery flecks (Mound and Kibby 1998). Damage in more restricted places can remain undetected until affected leaves mature or flowers develop and the damage becomes more apparent to the human eye (Childers 1997). Silvery appearance is often accompanied by faecal droplets which turn black as moulds grow on them (Childers 1997). Infestation with *T. tabaci* can lead to high yield losses through a reduction of photosynthetic activity and a disturbance of water retention (Nault and Shelton 2010). Damage is highest when thrips-feeding appears on meristematic tissues, because the tips of shoots can die back, reducing further growth (Moritz 2006; Emden 2013). Furthermore thrips feeding can lead to cosmetic damages such as blemished skin or discoloured petals in ornamental plants (Childers 1997; Kirk 1997). The host plant range of *T. tabaci* is wide and includes a variety of world-wide grown agricultural and horticultural crops such as cucumber, tobacco, tomato, potatoes, onion, leek, cabbage, sugar beet, cotton, olive trees, grape wine, peas etc. (Childers 1997; Alford 1999; Moritz 2006; Emden 2013).

In addition to the direct damage *T. tabaci* can cause to crops, it can also act as a vector for plant pathogenic viruses such as tomato spottet wilt virus (TSWV) and iris yellow spot virus (Mound 1996; Jones 2005; Diaz-Montano et al. 2010; Macharia et al. 2015). Furthermore thrips-damaged plant tissue is more vulnerable for entrance of bacterial or fungal pathogens such as the neck rot fungus (*Botrytis allii*) on onion (Childers 1997; Emden 2013).

3.3.5. Thrips rearing

T. tabaci individuals used in this research were obtained from a mass rearing on leek, at the Division of Plant Protection, University of Natural Resources and Life Sciences, Vienna. All thrips used in the experiments were thelytokous females from a parthenogenetically reproducing laboratory strain. Thrips were kept in glass jars containing pieces of leek to maintain feeding and oviposition. Additionally the bottom of the jars was covered with paper tissue to provide a hiding spot for the non-feeding stages of *T. tabaci*. The jars were closed using a fine mesh and a lid with a big hole to allow air circulation. The glass jars were kept in climate chambers at 25+/- 1 °C with a photoperiod of 16:8 (light/darkness) and 45 % relative air humidity.

3.4. Leaf disc bioassays

All bioassay were conducted with leaf discs punched from the cultivated cucumber plants (see 3.1. Test plant and application procedure), using cork borers with different diameters. Leaf discs were placed in glass Petri dishes on a thin film of 1 % water agar (Agar, Sigma-Aldrich, Vienna). Petri dishes with thrips were kept in a climate chamber at 25+/- 1 °C with a photoperiod of 16:8 (light/darkness) and 45 % relative air humidity.

3.4.1. Direct contact toxicity of treatments to 2nd instars

To evaluate the direct contact toxicity of the products to *T. tabaci*, a leaf disc bioassay adapted from Morishita (2001) and used as well by Peneder and Koschier (2011) was conducted. Leaf discs punched from untreated cucumber plants were placed on a thin layer of water agar (1 %) in a glass Petri dish (diameter 5 cm). Five adult *T. tabaci* females were randomly taken from the rearing site and put upon every leaf disc (diameter 2.2 cm) using a fine brush. Afterwards the Petri dish was closed with a plastic film (Cling Film, Roth, Germany). To allow air movement the film was perforated using insect pins (diameter 0.4 mm). The Petri dishes were

kept in the climate chamber for a 24h ovipositional period. Subsequently the insects were removed, the Petri dishes containing the leaf discs bearing the eggs, were again closed with a perforated cling film and kept in the climate chamber, where larvae could hatch from the eggs. On the sixth day, counted from the beginning of the experiment, the majority of the emerged larvae were 2nd instars and ready to be treated. Thus it took 5 days for *T. tabaci* to develop from egg to 2nd instar. For the spraying treatments the leaf discs with about 10 2nd instars on them were taken out of the Petri dishes and sprayed with 2.5 ml of the products mentioned above and with 2.5 ml water, using the Potter Spray Tower (Burkard Manufactoring Co Lt., Rickmansworth, UK). This apparatus has a nozzle that produces droplets in the aerosol and mist size range (< 100 µm diameter) and allows the treatment with a consistent measured dosage, resulting in a compound density on the leaf discs of about 1 µl/cm (Matthews 2000). After the treatment the leaf discs were returned to the Petri dishes again. Following this procedure the number of the alive larvae was counted and the Petri dishes were closed again. After 24 h and 48 h the number of dead and alive larvae was counted. The experiment was repeated 10 times.

3.4.2. Settling and oviposition preference

For this two choice assay, adapted from Peneder and Koschier (2011) and Egger and Koschier (2014) two leaf discs (diameter 1.5 cm) were placed opposite to each other on a film of water agar in a glass Petri dish cover (diameter 5.5 cm). One leaf disc was punched from a treated cucumber plant, the other from a cucumber plant treated with water (control). The distance between the two leaf discs was approximately 1.5 cm. The distance between the leaf discs and the edge of the Petri dish was about 0.5 cm. *T. tabaci* females of unknown age were transferred from rearing jars onto a Petri dish placed on ice to immobilize thrips to enable a faster transferring. Then 10 adult females were randomly collected and put in the centre be-

tween the two leaf discs using a fine brush. Afterwards the Petri dishes were closed with perforated plastic film and (after 30, 60, 120, 180, 300 and 360 minutes) the number of females on each leaf disc was counted. Adults located elsewhere in the petri dish (not on one of the leaf discs) were not counted. After 360 minutes the leaf discs were transferred to the climate chamber for 24 h and then the eggs laid on both leaf discs were counted under the binocular. The experiment was repeated 10 times.

3.4.3. Feeding and oviposition activity

For assessment of the feeding and ovipostion activity of *T. tabaci*, a leaf disc bioassay described by Peneder and Koschier (2011) was used. Leaf discs punched from the differently treated cucumber plants (diameter 1.1 cm) were put on a thin film of water agar. All female adults used in this experiment where synchronized by age i.e. 48 to 72 hours after emergence from pupal stage. Using the data describing the duration of *T. tabaci's* development stages published by van Rijn and co-authors (1995), this age should ensure that all female adults have overcome the pre-oviposition development stage that usually lasts about two days and are in the development stage with the highest ovipositional rate (van Rjin et al. 1995). A single adult female was transferred on one leaf disc using a fine brush. Subsequently the Petri dishes were closed with perforated plastic film and stored in the climate chamber. After 24h the adults were removed from the leaf discs and the feeding and ovipositional rate was evaluated. For evaluation of the feeding rate a transparent grid with 0.25 mm² fields was used to measure the surface area of damaged plant tissue under the binocular. For the ovipositional rate the eggs on the leaf disc were counted under the binocular. The experiment was repeated 25 times.

3.5. Histological characterisation

In order to check the cucumber leaves for histological changes, thin sections of leaf tissue were produced using Microtome and Virbatome.

For Vibratome sectioning small triangles were cut from the harvested cucumber leaves and dehydrated using ethanol in the following concentrations: 30 %, 50 %, 70 %, 90 %, 96 % for 4 minutes each at 4 °C. Afterwards the leaf pieces were put in a formaldehyd fixative and rinsed three times in 63 % ethanol for 10 minutes and one time in phosphate buffer for 10 minutes. The prepared leaf pieces were embedded in Low Melting Agarose (LMA; 5 % in phosphate buffer) (Sigma-Aldrich, USA). After hardening, the agarose blocks containing the leaf pieces were cut into small cubes with a scalpel. Subsequently 20 µm thin slices were prepared, using Vibratome (Leica VT1000S, Leica, Wetzlar, Germany). The leaf sections were than photographed at 40× magnification using an inverted light microscope (Axiovert 200M, Zeiss, Austria) with an integrated camera (Axio Cam MRc5, Zeiss, Austria). The thickness of the one cell layer thick leaf epidermis (Evert 2006) was measured with the program Axio vision rel. 4.6 (Zeiss, Austria).

For preparation of the leaf samples for Microtome sectioning a protocol used by Blaukopf and co-authors was adapted (Blaukopf et al. 2011). Small stripes of the harvested cucumber leaves were dehydrated using ethanol in the following concentrations: 30 %, 50 %, 70 %, 90 %, 96 % for 4 minutes each at 4 °C. Afterwards the leaf pieces were put in a paraformaldehyde-glutaraldehyd-fixative (100 mM phosphate buffer, pH 7.2, 2 % paraformaldehyde, 2.5 % glutaraldehyde) for several days. The fixative was removed, replaced by fresh fixative and subsequently vacuum was applied to the samples embedded in ice, for 15 minutes to enhance the penetration of the fixation. Subsequently the samples were rinsed three times in phosphate buffer (PBS) and embedded in LMA. Always 3 leaf stripes were placed in one block of LMA at a time. The blocks were transferred into paraformaldehyde-glutaraldehyd fixative, again vacuum was applied for 15 minutes to enhance fixation efficiency and the samples were incubated at 5 °C

over night. The LMA blocks containing the leaf samples were rinsed three times for 30 minutes in PBS (0.1 M, pH 7) and dehydrated using ethanol (30 %, 50 %, 70 %, 90 %, 96 % for 20 minutes). Each of the incubation steps was performed at 4 °C. Subsequently the ethanol was removed and 1 ml LR-White (Sigma-Aldrich, USA) was added to each LMA block. Vacuum was applied to the samples which were then incubated at 4 °C for 3 hours. The prepared blocks were sectioned using Microtome (Leica Ultracut R, Leica, Wetzlar Germany). This procedure includes trimming of the LR White blocks to a suitable sized form with a razor blade and then sectioning of the blocks into 2 µm thin sections. Afterwards the sectioned samples were stained for pectin, lignin and callose using protocols by Sobczak (1996). To visualize the structure of the cell wall Calcofluor was used, which stains cellulose and chitin. For staining of pectin, Rhutenium Red (Sigma-Aldrich, USA) was used. Samples were incubated 30 minutes with 0.2 % Rhutenium Red and afterwards rinsed with distilled water. For callose staining samples were incubated 5 minutes in Anilin Blue (Sigma-Aldrich, USA) and subsequently rinsed with distilled water. Lignin was stained using Phloroglucinol (Merck, Darmstadt,Germany). 0.2 g of Phloroglucinol was solved in 98 % ethanol and two parts of this solution (2 %) were mixed with one part concentrated HCL (36 %). Samples were stained for 30 min and rinsed with distilled water. For the cellulose/chitin staining to visualize the whole cell wall structure Calcofluor (Fluka, Sigma Aldrich,USA) was diluted 1:50 with distilled water. Sections were incubated for 10 min and afterwards rinsed with distilled water. Rhutenium Red and Phloroglucinol stained samples were examined using bright field microscopy where pectin and lignin were stained red, respectively Anilin Blue and Calcofluor stained samples were observed under UV light where callose and the whole cell wall were stained blue. Pictures of stained leaf sections were taken at 40× and 100× magnification using a light microscope (BX53, Olympus) with an integrated camera (XC50, Olympus). The software Olympus cellsense standard was used. For measurement of the thickness of epidermal cell layers and outer cell walls of epidermis cells the same software was used.

3.6. Statistical analyses

SPSS Statistics 21 was used for statistical analyses of the data. A confidence limit of 95 % was applied. Kolmogorov-Smirnoff and Levene tests were conducted to check data sets for normal distribution and homogeneity of variance. For the mortality rate a t-test for independent samples was used to compare the zero treatment and the water treatment. Afterwards independent sample t-tests were conducted to check for differences between treatments and water. No choice bioassay data was analysed with a one-way ANOVA and a Bonferroni post hoc test. Paired t-tests were conducted to check for differences within the settling and oviposition preference within the choice bioassay. The thickness of epidermal cells as well as of epidermal cell walls was compared between the treatments using a one-way ANOVA with a Games Howell post hoc test. All charts were created using Microsoft Excel 2013.

4. Results

4.1. Direct contact toxicity

To determine the direct contact toxicity of the plant strengthening products, first the data of the 24 h and 48 h mortality was compared between the water-treatment and the zero-treatment to exclude an effect of water on the mortality rate of *T. tabaci* using an independent sample t-test. Neither the 24 h mortality rate (t=-1.103; p=0.284) nor the 48 h mortality rate (t=-0.389; p=0.702) showed significant differences between the zero- and the water-treatment which means that water is not influencing the mortality rate of *T. tabaci* in this experiment. On basis of this result the water treatment could be compared to the *A. absinthium*, *T. vulgare* and "Pflanzen vital Spray S" treatments (Fig. 1).

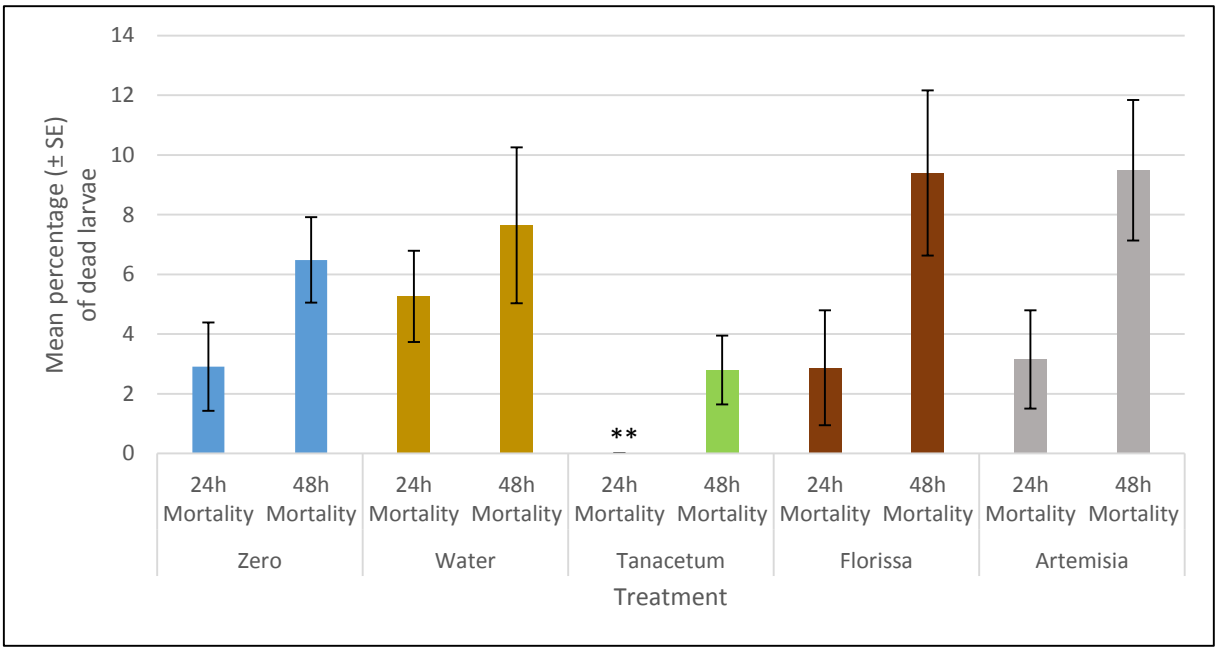


Figure 1: Mean percentage of dead *T. tabaci* 2nd instars (± SE) on cucumber leaf discs 24h and 48h after different treatments. Significant difference compared to water-treatment is indicated with * (p< 0.05).

A significant difference could be detected in the 24 h mortality rate between T. vulgare and water-treatment (t=3.434; p=0.007). In contrast, the mortality rate at 48 h showed no significant difference compared to water (t=1.701; p=0.106). The comparison between the *A. absinthium* and water treatment resulted in no significant differences in the 24 h (t=0.939; p=0.360) and

48h (t=-0.526; p=0.606) mortality rate. The same applies for the comparison between “Pflanzen vital Spray S” and water. Neither the 24 h (t=0.973; p=0.344) nor the 48 h (t=-0.461; p=0.650) mortality rate showed significant differences.

4.2. Settling and oviposition preference

The settling test with leaves from *A. absinthium* and water treated plants showed significant differences at 180 min (t=-3.226; p=0.007), 240 min (t=-3.144; p=0.008), 300 min (t=-3.771; p=0.003). At those times thrips adults preferred water treated leaves over *A. absinthium* treated leaves (Fig. 2).

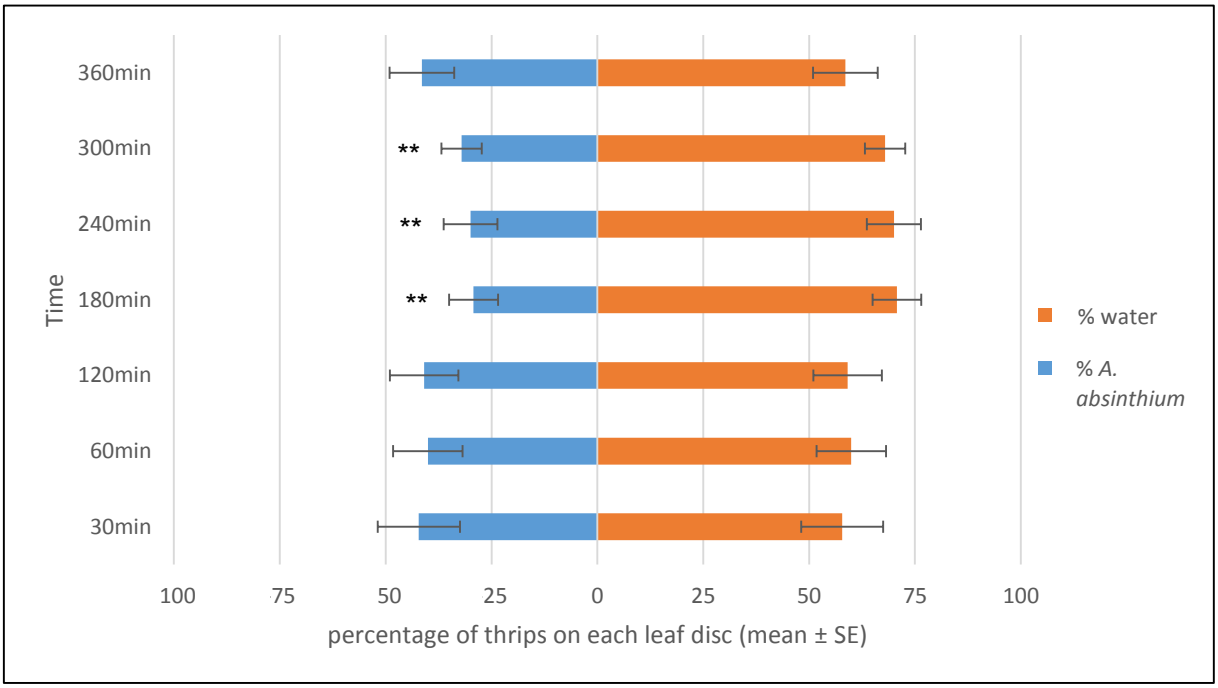


Figure 2: Mean percentage of *T. tabaci* adults (± SE) settled on leaf discs from cucumber plants treated with *A. absinthium* extract (treat) or with water (control) in a settlement preference test at 30 minutes, 1, 2, 3, 4, 5, 6 hours after release. Significant differences are indicated with ** (p< 0.01).

At the other times no significant differences in the settling preference could be detected. In general the mean numbers of *T. tabaci* adults which settled on leaf discs from water treated plants where higher than the number of adults which decided to settle on leaf discs of *A. ab-*

sinthium treated plants at every observation. Looking at the ovipositional preference no significant differences could be observed between *A. absinthium* and water treated leaves (t=-1.328; p=0.209;) (Fig. 3).

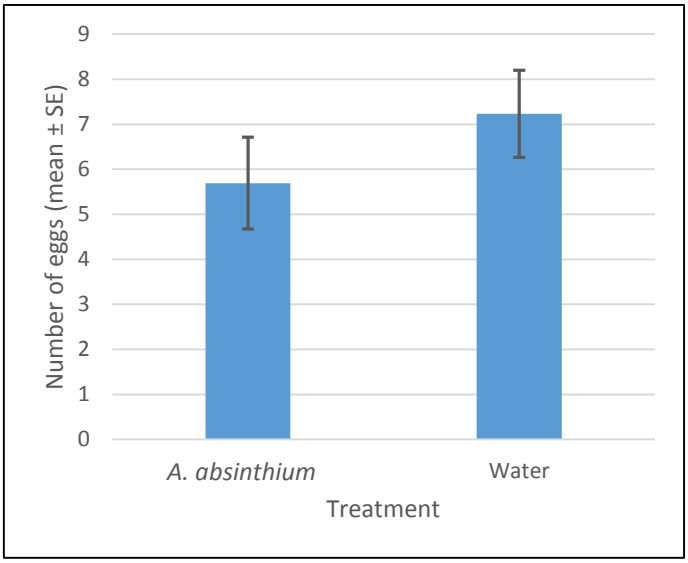


Figure 3: Mean percentage of eggs (± SE) laid on cucumber leaf discs treated with *A. absinthium* or water in a settling preference test.

The settling test with "Pflanzen vital Spray S" and water treated leaves showed significant differences at 30 min (t=-2.606; p=0,014) and 300 min (t=-0.014; p=0,021) (Fig. 4).

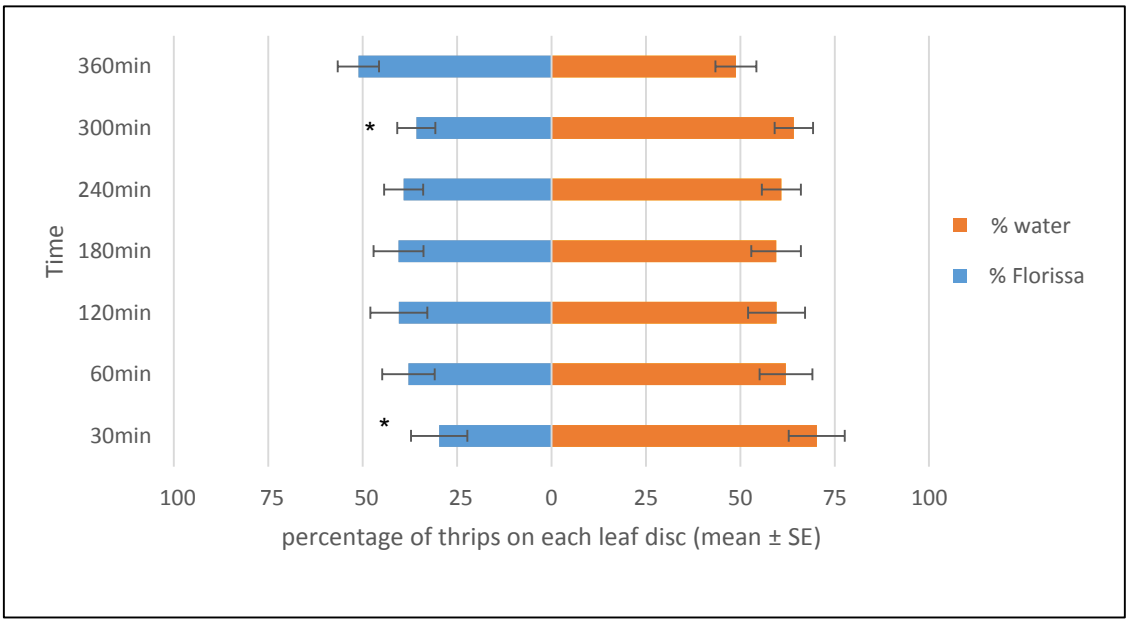


Figure 4: Mean percentage of *T. tabaci* adults (± SE) settled on leaf discs from cucumber plants treated with Pflanzen Vital Spray S (treat) or with water (control) in a settlement preference test at 30 minutes, 1, 2, 3, 4, 5, 6 hours after release. Significant differences are indicated with * (p< 0.05).

Thrips adults preferred leaves from water treated plants over leaves treated with the product. The other observations did not result in significant differences in the settling preference of *T. tabaci* between leaves harvested from differently treated cucumber plants. In general the mean numbers of *T. tabaci* adults which settled on leaf discs from water treated plants where higher than the number of adults which decided to settle on leaf discs of "Pflanzen vital spray S" treated plants at every observation, except the last. The ovipositional preference test resulted in a significance of p=0.51 (t=-2.132), indicating no significant ovipositional preference of *T. tabaci* between "Pflanzen vital Spray S" and water treated leaves (Fig. 5).

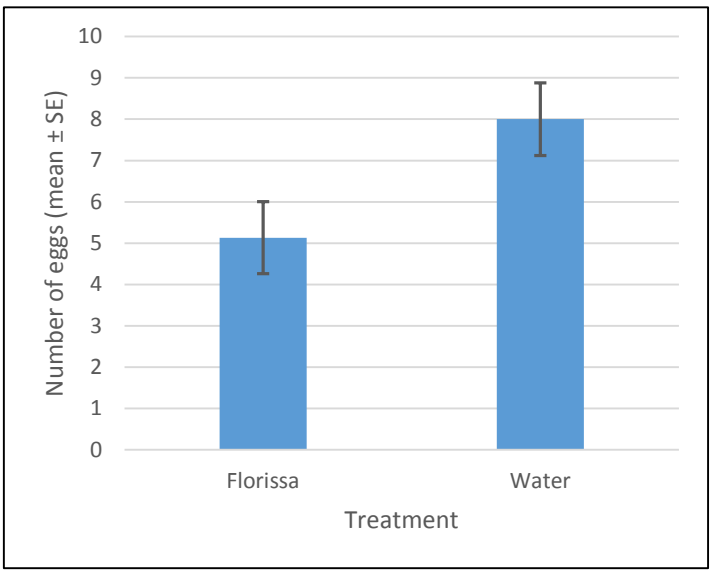


Figure 5: Mean percentage of eggs (± SE) laid on cucumber leaf discs treated with Florissa or water in a settling preference test.

The settling test with *T. vulgare* did not no result in any significant preferences of *T. tabaci* for either treatment or control (Fig. 6).

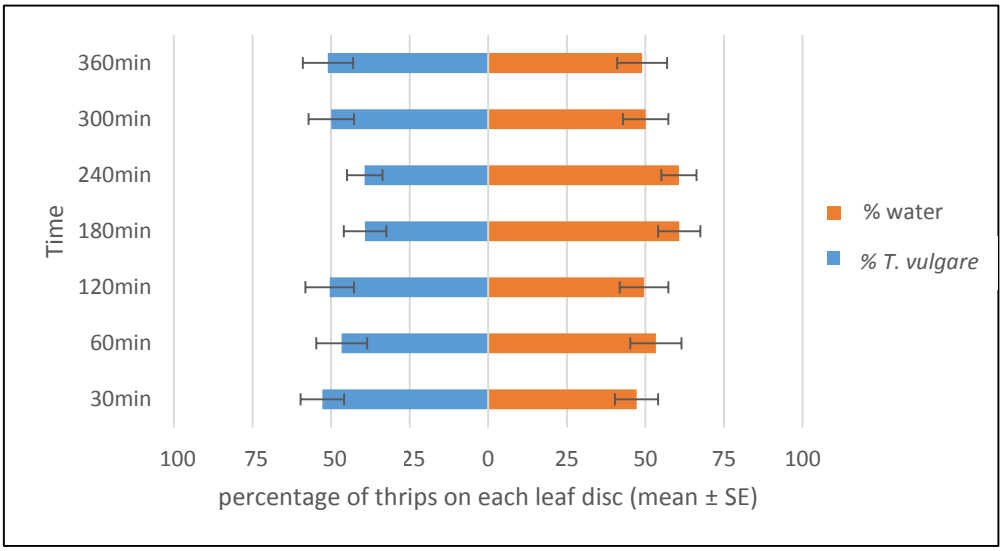
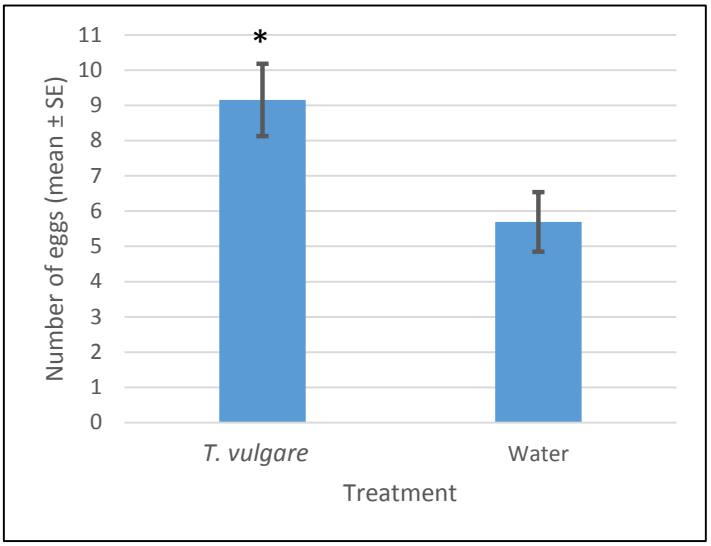


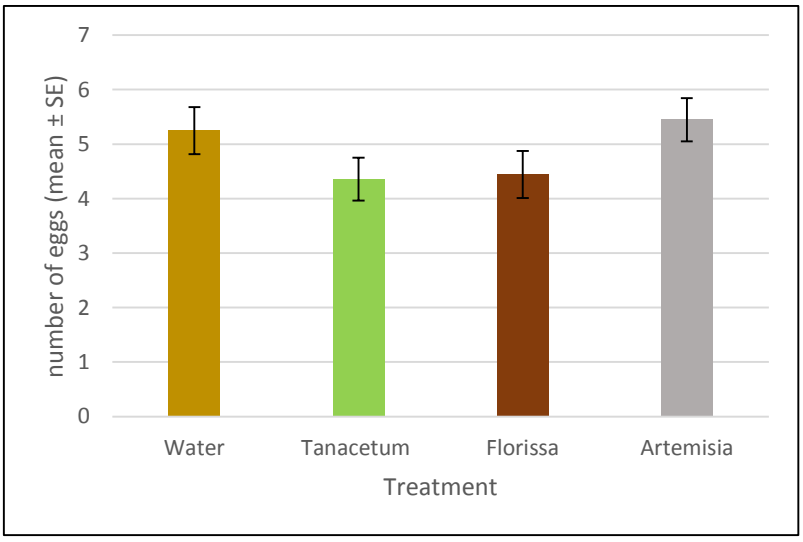
Figure 6: Mean percentage of *T. tabaci* adults (± SE) settled on leaf discs from cucumber plants treated with *T. vulgare* (treat) or with water (control) in a settlement preference test at 30 minutes, 1, 2, 3, 4, 5, 6 hours after release.



A significant difference could be detected in the ovipostional preference of thrips between leaves harvested from water and *T. vulgare* treated cucumber plants (Fig. 7). Thrips adults significantly preferred to lay eggs on leaves of plants treated with *T. vulgare* (t=2.320; p=0.039).

Figure 7: Mean percentage of eggs (± SE) laid on cucumber leaf discs treated with *T. vulgare* or water in a settling preference test. Significant difference compared to water-treatment is indicated with * (p< 0.05).

4.3. Feeding and oviposition rate



A one way ANOVA of data collected from the no choice oviposition test showed no significant differences in the oviposition rate of *T. tabaci* adults on leaf discs punched from water, *T. vulgare*, *A. absinthium* or "Pflanzen vital Spray S" treated cucumber plants (F=1.814; df=3; p=0.149) (Fig. 8).

Figure 8: Mean number of eggs (± SE) on leaf discs harvested from cucumber plants, treated with water, *T. vulgare,* "Pflanzen vital Spray S" (=Florissa) or *A. absinthium* in a no choice test situation.

Leaf discs from *T. vulgare* and “Pflanzen vital spray S” treated plants resulted in lower mean numbers of eggs than leaf discs from water treated plants however this difference is not significant.

ANOVA analysis of the feeding damage data showed no significant differences in the feeding damage of *T. tabaci* adults between leaf discs punched from differently treated cucumber plants (F=1.066; df=3; p=0.367). Leaf discs from *T. vulgare*, *A. absinthium* and “Pflanzen vital spray S” treated plants resulted in lower mean numbers of eggs than leaf discs from water treated plants however this difference is not significant (Fig. 9).

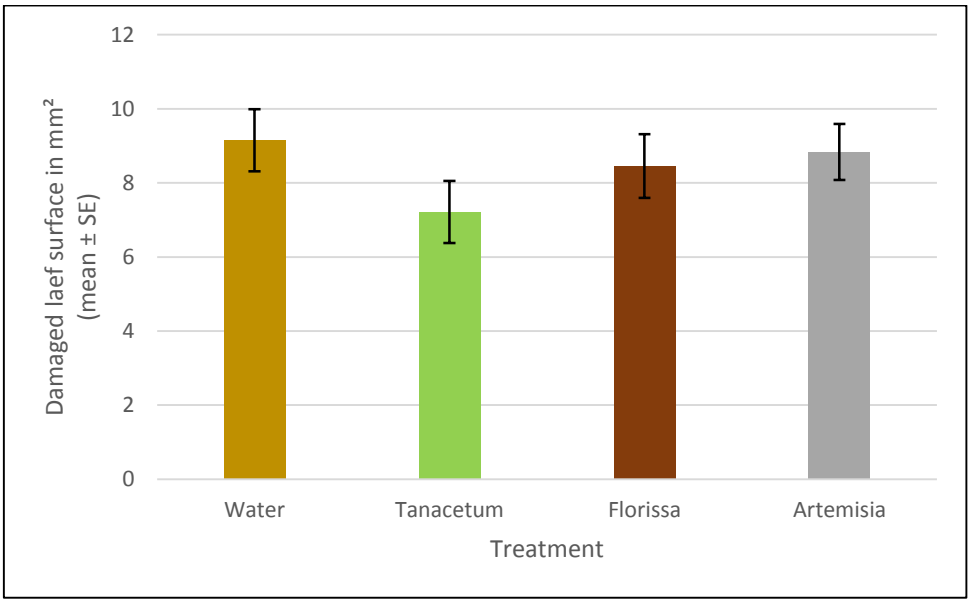


Figure 9: Damaged leaf surface (in mm², mean ± SE) of leave discs harvested from cucumber plants treated with with water, *T. vulgare,* “Pflanzen vital Spray S” (=Florissa) or *A. absinthium* in a no choice test situation.

4.4. Histological characterisation

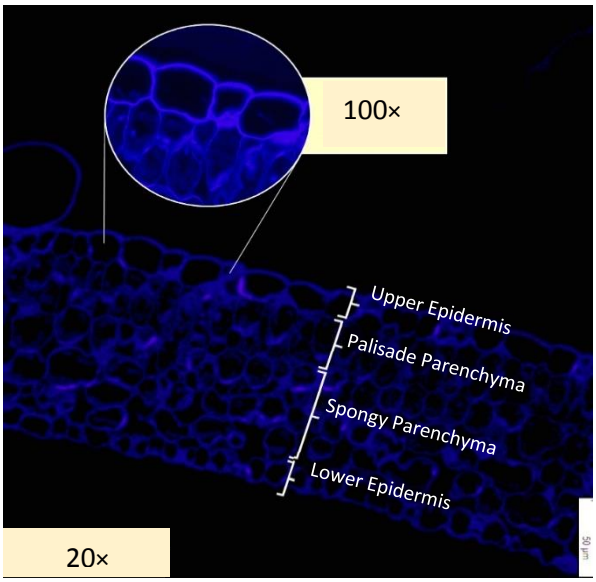


Figure 10: 2 µm thick cucumber leaf cross section stained with Calcofluor and photographed with 20× magnification with an UV filter. Inset shows a detailed picture of upper epidermis photographed with 100× magnification.

To allow a better understanding of the following results and to give an overview of the structure of a leaf cross section in principal, a Calcofluor stained leaf section was photographed at 20× and 100× magnification. Using this pictures, a scheme was made that provides the reader with basic information about the structure of a leaf (Fig. 10).

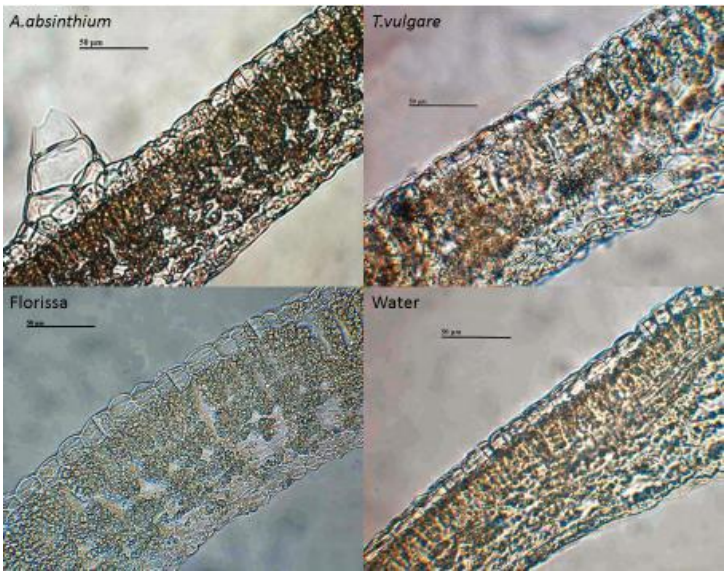


Figure 11: 20 µm thick Vibratome cross sections of cucumber leaves treated as indicated in the figures. The pictures were taken at 40× magnification using inverse light microscopy.

ANOVA analysis of results obtained from Vibratome sectioned leaves (Fig. 11) showed significant differences between the groups ($F=3.862$; $df=3$; $p=0.010$). A Games Howell post hoc test could detect significant differences in the thickness of the epidermal cell layer between the water control and both *T. vulgare* ($P=0.006$) and *A. absinthium* ($P=0.007$) treated leaves (Table 1). Between leaves treated with water and leaves treated with “Pflanzen vital Spray S”

no significant difference could be detected. However the average thickness of the epidermis cells is lower than the one of water-treated pants.

Treatment	Mean	Std. Error	Sig. (p value)
Water	10,9753 µm	0,28741	
A. absinthium	12,3535 µm	0,30666	0,007 **
T. vulgare	12,3436 µm	0,29737	0,006 **
Florissa	12,3706 µm	0,48190	0,067 n.s.

Table 1: ANOVA results: Mean thickness of upper epidermis cells (µm) of cucumber leaves treated with plant strengthening products and water. With std. error and p value (Games Howell post Hoc test).

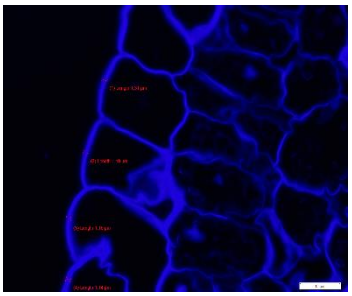


Figure 12: 2 µm thick Microtome cross section of a cucumber leaf. Red bars indicate measurements of the cell walls thickness.

Analysis of the cell wall thickness of the upper epidermis cells (Fig. 12) shows that all three products resulted in a significant thicker cell wall of cucumber leaves compared to water plants. The Games Howell post hoc test showed that leaves harvested from plants treated with A. absinthium (p= 0.000), T. vulgare (p= 0.000) or “Pflanzen vital Spray S” (p= 0.034) had thicker cell walls in the upper epidermis compared to leaves from water-treated cucumber plants (see Table 2).

Treatment	Mean	Std. Error	Sig. (p value)
Water	0,5855 µm	0,0167	
A. absinthium	0,7676 µm	0,0279	0,000***
T. vulgare	0,7437 µm	0,0205	0,000***
Florissa	0,6557 µm	0,0192	0,034*

Table 2: ANOVA results: Mean thickness of cell walls of upper epidermis cells (µm) of cucumber leaves treated with plant strengthening products and water with std. error and p value (Games Howell post Hoc test).

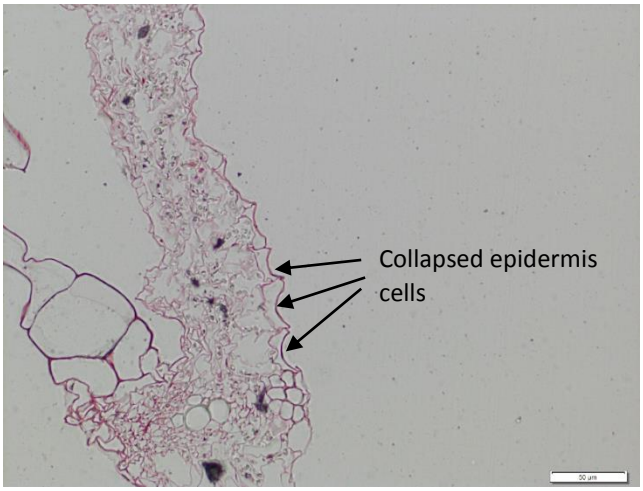


Figure 13: 2 µm thick Microtome cross section of cucumber leaf stained with Ruthenium Red.

The leaf sections produced with Microtome could not be used for measuring the thickness of the whole epidermis cells (as done with Vibratome sectioned leaves) because some sections did not exhibit an intact tissue (Fig. 13). This would distort the results because the collapsed cells are much smaller in diameter compared to intact epidermis cells.

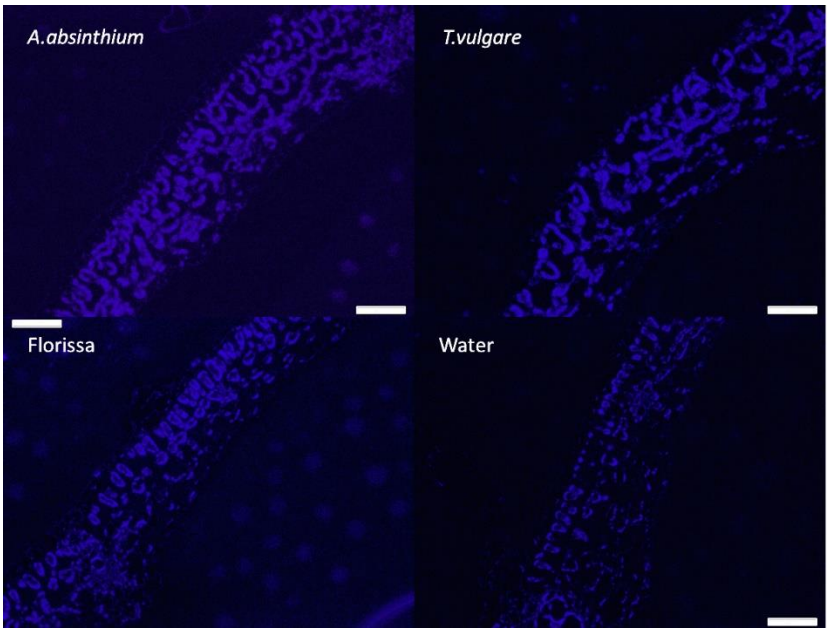
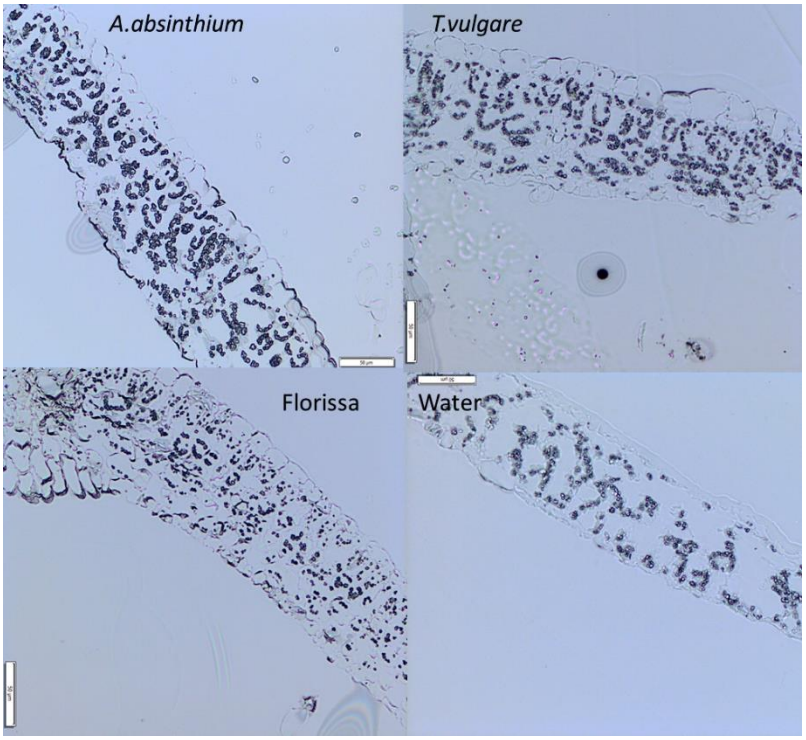


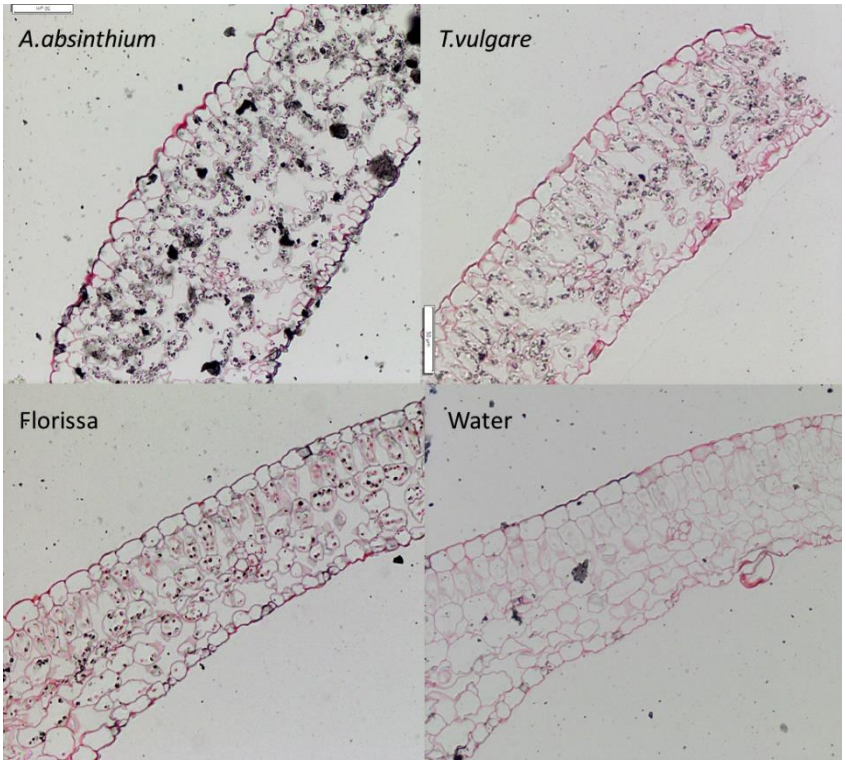
Figure 14: 2 µm thick Microtome cross sections of cucumber leaves treated as indicated in the pictures. Callose is stained with Anilin Blue and appears blue. The pictures were taken at 20× magnification using light microscopy with UV filter.

Staining of callose with Anilin Blue resulted in a visible blue staining in all four treatments (Fig. 14). It was noticeable that no blue staining could be detected in the epidermis layer, which can be seen on the upper side of the leaf sections in figure 11, but mainly in the palisade parenchyma. No difference could be observed between the treatments.



In all of the treatments staining of lignin with Phloroglucinol did not result in any visible red staining (Fig. 15). No differences could be observed between the treatments.

Figure 15: 2 µm thick Microtome cross sections of cucumber leaves treated as indicated in the pictures. Lignin is stained with Phloroglucinol and should appear red. The pictures were taken at 20× magnification using light microscopy.



Staining of pectin with Ruthenium Red resulted in a visible red staining in cell walls specifically in the upper epidermis (on the upper side of the leaf sections in figure 16). It seems that cell walls of the water treated leaves did not appear as red as cell walls of plant strengthening product treated ones.

Figure 16: 2 µm thick Microtome cross sections of cucumber leaves treated as indicated in the pictures. Pectin is stained with Ruthenium Red and appears red. The pictures were taken at 20× magnification using light microscopy.

4.5. Summary

Table 3 summarizes the results in order to give a better overview on all three tested products.

	A. absinthium	*T. vulgare*	Florissa
Mortality rate 24/48h	0/0	-**/0	0/0
Settlement preference	-**	0	-*
Oviposition preference	0	+*	0
Oviposition rate	0	0	0
Feeding damage	0	0	0
Epidermis cells	+**	+**	0
Epidermal cell walls	+***	+***	+*
Callose staining	0	0	0
Lignin staining	0	0	0
Pectin staining	+	+	+

Table 3: Summarized results. All 3 tested products are listed with the respective results of the conducted tests. 0 = no significant increase/decrease in relation to the water-control. + = increase in relation to water-control. - = decrease in relation to water-control. *($p< 0.05$), **($p< 0.01$) and ***($p< 0.001$) indicate the levels of significance.

5. Discussion

Direct contact toxicity

Results of the first bioassay show that all tested plant strengthening products do not have a direct contact toxicity to *T. tabaci* 2nd instars. None of the plant strengthening product-treatments resulted in a significantly higher 24h or 48h mortality rate of 2nd instars compared to the water-treatment. Thus - from a legal point of view - the outcome of the experiment supports the classification of the products as “plant strengtheners” in contrast to “plant protection agents”. None of the tested products exhibited a direct toxic effect on the pest itself, which would classify them as plant protection agents according to Austrian legislation (BMEL-Pflanzenschutzgesetz). The treatment with *T. vulgare* even resulted in a significantly lower 24h mortality rate of *T. tabaci* compared to the water-treatment. When considering that *T. vulgare* extract is classified as irritant, the reason for the reduced 24h mortality is unclear, and a repetition of the test should be conducted.

A. absinthium extract (used in this research) seems to have a lower toxicity than *A. absinthium* essential oil which reportedly has contact toxicity against arthropods such as *Tetranychus urticae* (Koch); *Oryzaephilus surinamensis* L. and *Tribolium castaneum* (Herbst) (Chiasson et al. 2001; Bachrouch et al 2015). The same applies for *T. vulgare* essential oil which has been proven to have acaricidal effects (Chiasson et al. 2001).

Production of 2nd instars used in this experiment led to the observation that *T. tabaci* needed 5 days to develop from egg to 2nd instar. When comparing this outcome with the development time published by van Rijn and co-authors (1995), which is 6.05 ± 0.77 from egg to 2nd instar it can be noted that the development time in this research approximately matches the development time described by van Rijn and co-authors.

Settling and oviposition preference

The data shows that *T. tabaci* prefers to settle on leaves of water treated plants over leaves from *A. absinthium* and “Pflanzen vital Spray S” treated plants, meaning that *A. absinthium* and “Pflanzen vital Spray S” have a repellent effect on the settling preference of *T. tabaci*. In contrast no significant preference to settle on leaves of water or *T. vulgare* treated plants could be detected, which indicates that *T. vulgare* does not have a repellent effect on the settlement decision of *T. tabaci* adults. Ovipositional preference data shows that *A. absinthium* and “Pflanzen vital Spray S” do not have a deterrent effect on the oviposition of *T. tabaci. T. vulgare* even stimulates the oviposition. Because the number of thrips settling on leaf discs harvested from water treated plants was never significantly higher at the last measuring time, compared to the number of thrips settling on leaf discs from *A. absinthium* and “Pflanzen vital Spray S” treated plants and additionally *A. absinthium* and “Pflanzen vital Spray S” did not deter thrips to lay eggs on leaves of treated plants, it becomes obvious that *A. absinthium* and “Pflanzenvital Spray S” do not negatively affect the host plant preference of *T. tabaci*. The preference of thrips to lay eggs on leaves of *T. vulgare* treated plants can be deducted as a ovipositional preference for “strengthened” plant tissue when they are not previously repelled to settle on them (as in case of *A. absinthium* and “Pflanzenvital Spray S”). The reason for this preference could be that “strengthened” plant tissue could potentially provide a better spot for the deposition of eggs, which are vulnerable to desiccation and cannot defend themselves against predators and parasites (Kirk 1997). This outcome also matches with the histological characterisation results of leaves from *T. vulgare* treated plants that show thicker epidermis cells and thicker epidermis cell walls compared to water treated plants (see histological characterisation). The interpretation that *T. tabaci* could prefer to lay eggs into leaves with thicker epidermis cells because those provide better embedding and protection for the eggs could be an explanation for the present results. Further testing should also include older plants that have been treated more often to potentially increase the observed effects.

When looking at the outcome of the settling preference test, the question arises if the observed settling preference for leaves of water treated plants over leaves from *A. absinthium* and "Pflanzen vital Spray S" treated plants is due to an product-induced plant response - which would mean a real plant strengthening effect - or if it is an effect of residues of the plant strengthening products on the leaf surface which could have a repellent effect themselves. In the first case it could be possible that the tested products induced the cucumber plants to build up chemical or structural defences. This defences could then influence the settling preference of *T. tabaci* on cucumber. Anatomical-structural defences that could possibly influence the settling of insects, are mainly an increased leaf-trichome formation (Dalin et al. 2008) or changes in the physiomechanical and chemical function of the cuticle (Müller 2008). However until now these defences were only reported to be induced in plants after herbivory attack and not as a result of plant strengthening agents. Additionally *T. tabaci* is, at least in case of cotton, not affected by trichomes on leaves (Kirk 1997).

Chemical defences could include the production of secondary metabolites that act repellent on thrips. A plant strengthener called "Milsana" reportedly elicits the biosynthesis of polyphenol flavonoids and therefore protects cucumber against powdery mildew (Fofana et al. 2002). Rühmann could prove the induction of resveratrol derivates (also polyphenols) in *Vitis vinifera* callus cultures with the yeast *Aureobasidium pullulans* (Rühmann 2005) and Chitosan treatments of rice also resulted in the production of phytoalexins (Agrawal et al. 2002). Even if most research about defence related secondary metabolites (phytoalexins) is carried out in relation to pathogen defence, the above listed examples show that the plant's secondary metabolism - which is known as a key factor in the defence system of plants (Engelberth et al. 2010) - can be influenced by external stimuli and this could also be true in the case of application of *A.absinthium* and *T. vulgare* on plants. When focusing on insects there is proof that certain phytoalexins which are mainly tannins and terpenoids are involved in the defence of herbivory attack (Forkner et al. 2004; Barbehenn et al. 2006; Moctezuma et al. 2014). Many volatile terpenoids such as pyrethroids or limonoids are reported to have toxic and repellent effects on arthropods

(Engelberth et al. 2010; Kant et al. 2015). However induced physiological and anatomical effects of plant strengthening products on crop plants are rarely studied, especially against herbivores. Further research could evaluate if there are any differences in the thickness of the cuticle or the trichome density between the leaves of differently treated plants. Additionally the amount of relevant phytoalexins in the leaf tissue could be analysed using e.g. high pressure liquid chromatography (HPLC).

In the case of the product's residues causing the differences in the settling preference of *T. tabaci,* the product itself would have a repellent effect on the settling of *T. tabaci*. Effects of different chemical and natural substances on the settling preference of thrips are well-studied. Egger and Koschier (2014) could verify a repellent effect of methyl jasmonate against *T. tabaci* in the same two choice situation used in this research. Martinez and co-authors (2015) could verify a repellent effect of *A. absinthium* extract against *Demotispa neivai* Bondar and Halbert and co-authors (2009) could verify a repellent effect of *A. absinthium* essential oils against aphids. The insect repellent activity of *A. absinthium* residues could be a reason why *T. tabaci* did prefer to settle on water treated leaves over *A. absinthium* and "Pflanzen vital Spray S" (which also contains *A. absinthium* extract) leaves. Interestingly *T. vulgare* was used by Native Americans as an insect repellent (Chiasson 2001). Despite this, the settling test did not result in repellent effect on *T. tabaci* and as said before the oviposition was even higher on *T. vulgare* treated leaves.

Feeding and oviposition rate

The no choice bioassays reveal that the plant strengthening effect of all 3 products against *T. tabaci* on cucumber is low when measurable. No significant decline in the feeding and ovipositional rate of *T. tabaci* on leaves of product treated plants could be observed compared to leaves of water treated plants. Thus it can be reasoned that *A. absinthium*, *T. vulgare* and "Pflanzen vital spray S" do not have a deterrent effect on the feeding and ovipositional rate of

T. tabaci on cucumber. One disadvantage of no-choice tests in general is that insects cannot choose between different treatments. It could be possible that insects would show different behaviour under a choice situation. This circumstance was also observed by Pobozniak and Koschier (2014) with *T. tabaci* and different pea cultivars. They obtained different results under choice and no choice situations. However it has to be taken into account that in the choice bioassay used in this study the ovipositional rate was never lower on leaves of water treated plants in comparison to leaves of product treatment plants.

Histological characterisation

The histological characterisation of Vibratome sectioned leaves showed that the upper epidermis cell layer was thicker in case of *A. absinthium* and *T. vulgare* treated plants compared to water-treated plants. Additionally Microtome-sectioned leaves from *A. absinthium* and *T. vulgare* and "Pflanzen vital Spray S"-treated cucumber plants showed thicker epidermal cell walls compared to water treated plants. Thus *A. absinthium* and *T. vulgare* and to a lower extent "Pflanzen vital Spray S" do have an influence on the epidermal cell wall structure of cucumber leaves. The epidermis is known to protect the leaf tissue from mechanical damage and the entry of organisms (Beck 2005; Evert 2006). Moreover leaf toughness reportedly affects the penetration of plant tissues by mouthparts of piercing sucking insects (Howe et al. 2008) and cell walls are responsible for the mechanical strength of plant structures (Taiz et al. 2010). Therefore it could be speculated, that plants with a thicker leaf epidermis containing thicker cell walls are better protected and less susceptible to piercing sucking insects like *T. tabaci*, that needs to penetrate through the outer epidermal cell wall (Kirk 1997). However this "strengthening effect" could not result in a significant reduction of the feeding and oviposition rate of *T. tabaci* in under no choice conditions in this study.

By staining Microtome sectioned leaves for lignin, no coloration could be detected, which leads to the conclusion that no lignin was present in the leaf tissue. This is not surprising because

lignin is normally only present in secondary cell walls, where it is relevant for rendering the wall waterproof and stronger (Cosgrove 2010; Albersheim et al. 2011). The plant strengthening products did not elicit a lignin biosynthesis neither in the epidermis cells nor anywhere else in the leaf tissue. When looking at the pectin staining it could be observed that leaf sections obtained from water treated plants had less red coloration than leaf sections from plant strengthening product-treated plants. Since pectin, besides cellulose, is one of the main constituents of the plant's cell wall (Albersheim et al. 2011) and supposed to form a gel phase for the embedding of the cellulose-hemicellulose network, this result matches with the epidermis cell wall thickness data which showed thicker cell walls (which also means more pectin in total) in case of product treated leaves. Staining of callose did not result in any visible differences between the treatments leading to the conclusion that the callose biosynthesis is not influenced by the plant strengthening products.

6. Conclusion

Interpretation of the obtained results from a plant protection point of view leads to the conclusion that extracts of *A. absinthium* and *T. vulgare* as well as "Pflanzen vital Spray S", are not suitable to "strengthen" cucumber plants leading to a measurable increased resistance against *T. tabaci*.

The avoidance of *T. tabaci* to settle on leaves of *A. absinthium* and "Pflanzen vital Spray S" treated cucumber plants is not relevant in terms of plant protection because it did not result in a reduced host plant preference or ovipositional preference. This indicates that there is the same leaf damage through the ovipositional activity of *T. tabaci* on water treated compared to product treated leaves and additionally also the amount of progeny would be the same and in case of *T. vulgare* even more.

Also the thicker epidermis cells of leaves of *A. absinthium* and "Pflanzen vital Spray S" treated plants and thicker epidermis cell walls of leaves from *T. vulgare, A. absinthium and* "Pflanzen

vital Spray S" treated plants did not result in a reduced feeding and ovipositional rate in the no choice bioassays and are therefore not relevant in terms of protecting plants against *T. tabaci* damage.

Considering the circumstance that all plant protection relevant parameters studied here (ovipositional preference, feeding damage and oviposition rate), did not show significant differences between water treated and product treated plants, the claimed strengthening effect against sucking insects of the products could not be verified in this research in case of *T. tabaci* and cucumber.

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Annex: Data Tables

Direct contact toxicity

	0 Control		Water		Tanacetum vulgare		Artemisia absinthium		Pflanzen vital Spary S	
	Leaf disc number	Number of living females	Leaf disc number	Number of living females	Leaf disc number	Number of living females	Leaf disc number	Number of living females	Leaf disc number	Number of living females
After treatment	1	12	1	12	1	11	1	15	1	9
	2	10	2	14	2	15	2	11	2	14
	3	10	3	11	3	12	3	10	3	11
	4	10	4	12	4	11	4	13	4	13
	5	11	5	12	5	9	5	9	5	15
	6	12	6	10	6	14	6	11	6	8
	7	12	7	15	7	12	7	9	7	12
	8	11	8	16	8	13	8	13	8	10
	9	11	9	15	9	15	9	11	9	14
	10	14	10	14	10	16	10	9	10	16
after 24h	1	12	1	11	1	11	1	13	1	9
	2	9	2	13	2	15	2	10	2	14
	3	10	3	11	3	12	3	10	3	11
	4	9	4	11	4	11	4	13	4	11
	5	10	5	11	5	9	5	9	5	13
	6	12	6	10	6	14	6	10	6	8
	7	12	7	15	7	12	7	9	7	12
	8	11	8	16	8	13	8	13	8	10
	9	11	9	13	9	15	9	11	9	14
	10	14	10	13	10	16	10	9	10	16
after 48h	1	11	1	9	1	11	1	12	1	8
	2	9	2	13	2	14	2	9	2	13
	3	9	3	11	3	11	3	9	3	9
	4	9	4	11	4	11	4	13	4	11
	5	10	5	11	5	9	5	8	5	11
	6	11	6	10	6	14	6	10	6	8
	7	12	7	15	7	12	7	9	7	11
	8	10	8	16	8	13	8	11	8	10
	9	11	9	13	9	14	9	11	9	13
	10	14	10	12	10	15	10	8	10	16

Settling Preference

Species	Thrips tabaci	**Plant**	Cucumber "Korinda"
Treatment	Artemisia absinthium	**Nr. individuals / unit**	10

30 min

rep.	treat	control	elsewhere
1	2	1	7
2	0	5	5
3	1	5	4
4	6	3	1
5	3	4	3

60 min

rep.	treat	control	elsewhere
1	3	3	4
2	1	2	7
3	1	5	3
4	5	4	1
5	3	3	4

240 min.

rep.	treat	control	elsewhere
1	1	4	5
2	1	5	4
3	2	6	2
4	4	3	3
5	2	8	0

120 min.

rep.	treat	control	elsewhere
1	3	5	2
2	0	4	6
3	2	5	3
4	7	3	0
5	6	3	1

300 min.

rep.	treat	control	elsewhere
1	1	5	4
2	3	4	3
3	1	5	3
4	3	3	4
5	4	5	1

180 min.

rep.	treat	control	elsewhere
1	2	5	3
2	0	4	6
3	3	3	4
4	5	4	1
5	3	7	0

360 min.

rep.	treat	control	elsewhere
1	3	5	2
2	1	4	5
3	3	4	3
4	6	1	3
5	2	4	4

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Artemisia absinthium

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	0	1	9
2	1	0	9
3	1	4	5
4	0	3	7
5	1	6	3

60 min

rep.	treat	control	elsewhere
1	0	1	9
2	1	0	9
3	1	4	5
4	0	3	7
5	0	6	4

120 min.

rep.	treat	control	elsewhere
1	0	0	10
2	1	0	9
3	0	2	8
4	0	3	7
5	1	5	4

180 min.

rep.	treat	control	elsewhere
1	0	0	10
2	0	1	9
3	2	5	3
4	0	1	9
5	1	6	3

240 min.

rep.	treat	control	elsewhere
1	0	0	10
2	0	1	9
3	3	3	4
4	0	1	9
5	0	6	4

300 min.

rep.	treat	control	elsewhere
1	0	0	10
2	0	0	10
3	2	3	5
4	0	0	10
5	0	7	3

360 min.

rep.	treat	control	elsewhere
1	1	0	9
2	0	0	10
3	2	2	6
4	0	2	8
5	0	6	4

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Artemisia absinthium

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	8	1	1
2	4	2	4
3	8	0	2
4	0	10	0
5	5	5	0

60 min

rep.	treat	control	elsewhere
1	5	3	2
2	4	4	2
3	9	1	0
4	1	7	2
5	6	4	0

120 min.

rep.	treat	control	elsewhere
1	4	4	2
2	3	5	2
3	4	4	2
4	5	2	3
5	4	5	1

180 min.

rep.	treat	control	elsewhere
1	5	3	2
2	3	3	4
3	4	4	2
4	1	4	5
5	2	8	0

240 min.

rep.	treat	control	elsewhere
1	5	3	2
2	3	2	5
3	4	3	3
4	2	3	5
5	1	8	1

300 min.

rep.	treat	control	elsewhere
1	5	4	1
2	3	4	3
3	2	6	2
4	2	5	3
5	2	7	1

360 min.

rep.	treat	control	elsewhere
1	5	2	3
2	2	7	1
3	2	5	3
4	2	5	3
5	6	4	0

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Pflanzen Vital Spary S

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	3	4	3
2	2	4	4
3	1	6	3
4	4	4	2
5	0	8	2

60 min

rep.	treat	control	elsewhere
1	3	5	2
2	5	5	0
3	0	8	2
4	3	5	2
5	2	3	5

120 min.

rep.	treat	control	elsewhere
1	3	3	4
2	2	8	0
3	0	8	2
4	4	5	1
5	1	4	5

180 min.

rep.	treat	control	elsewhere
1	4	4	2
2	2	7	1
3	0	8	2
4	4	5	1
5	3	5	2

240 min.

rep.	treat	control	elsewhere
1	4	4	2
2	3	5	2
3	1	7	2
4	4	3	3
5	4	6	0

300 min.

rep.	treat	control	elsewhere
1	5	1	4
2	1	5	4
3	0	8	2
4	4	4	2
5	4	4	2

360 min.

rep.	treat	control	elsewhere
1	4	3	3
2	2	4	4
3	1	6	3
4	2	1	7
5	3	2	5

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Pflanzen Vital Spary S

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	3	3	4
2	1	6	3
3	1	0	9
4	0	2	8
5	0	3	7

60 min

rep.	treat	control	elsewhere
1	3	4	3
2	2	5	3
3	1	0	9
4	0	2	8
5	1	4	5

120 min.

rep.	treat	control	elsewhere
1	4	4	2
2	0	5	5
3	1	0	9
4	1	1	8
5	1	3	6

180 min.

rep.	treat	control	elsewhere
1	4	4	2
2	2	2	6
3	2	0	8
4	0	2	8
5	1	3	6

240 min.

rep.	treat	control	elsewhere
1	3	4	3
2	2	3	5
3	0	1	9
4	2	1	7
5	1	1	8

300 min.

rep.	treat	control	elsewhere
1	2	4	4
2	2	3	5
3	1	1	8
4	1	2	7
5	1	2	7

360 min.

rep.	treat	control	elsewhere
1	5	3	2
2	2	6	2
3	3	1	6
4	3	4	3
5	2	2	6

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Pflanzen Vital Spary S

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	0	10	0
2	3	7	0
3	0	9	1
4	4	6	0
5	5	2	3

60 min

rep.	treat	control	elsewhere
1	0	10	0
2	4	6	0
3	3	4	3
4	6	3	1
5	5	3	2

120 min.

rep.	treat	control	elsewhere
1	1	8	1
2	3	7	0
3	6	1	3
4	5	3	2
5	4	3	3

180 min.

rep.	treat	control	elsewhere
1	3	6	1
2	3	7	0
3	6	2	2
4	4	6	0
5	4	4	6

240 min.

rep.	treat	control	elsewhere
1	1	8	1
2	2	6	2
3	5	2	3
4	4	6	0
5	3	4	3

300 min.

rep.	treat	control	elsewhere
1	3	6	1
2	2	7	1
3	3	5	2
4	1	8	1
5	3	4	3

360 min.

rep.	treat	control	elsewhere
1	3	5	1
2	5	4	1
3	3	3	4
4	3	5	2
5	3	0	7

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Tanacetum vulgare

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	2	2	6
2	2	1	7
3	1	2	7
4	5	2	3
5	1	1	8

60 min

rep.	treat	control	elsewhere
1	2	2	6
2	3	2	5
3	3	3	4
4	4	1	5
5	6	6	4

120 min.

rep.	treat	control	elsewhere
1	3	4	3
2	3	5	2
3	4	2	4
4	4	1	5
5	0	7	3

180 min.

rep.	treat	control	elsewhere
1	4	3	3
2	3	5	2
3	4	4	2
4	4	1	5
5	2	5	3

240 min.

rep.	treat	control	elsewhere
1	0	3	7
2	4	4	2
3	4	4	2
4	3	2	5
5	2	4	4

300 min.

rep.	treat	control	elsewhere
1	2	5	3
2	5	1	4
3	4	2	4
4	5	0	5
5	4	3	3

360 min.

rep.	treat	control	elsewhere
1	2	6	2
2	4	2	4
3	6	2	2
4	4	1	5
5	3	5	2

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Tanacetum vulgare

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	0	0	10
2	0	3	7
3	2	1	7
4	1	1	8
5	7	1	2

60 min

rep.	treat	control	elsewhere
1	0	0	10
2	0	1	9
3	2	1	7
4	0	1	9
5	4	1	5

120 min.

rep.	treat	control	elsewhere
1	0	0	10
2	1	5	4
3	3	0	7
4	1	0	9
5	3	2	5

180 min.

rep.	treat	control	elsewhere
1	1	2	7
2	1	4	5
3	1	3	6
4	0	2	8
5	5	0	5

240 min.

rep.	treat	control	elsewhere
1	3	1	6
2	1	3	6
3	3	2	5
4	0	2	8
5	2	2	7

300 min.

rep.	treat	control	elsewhere
1	3	2	5
2	1	4	5
3	2	2	6
4	0	3	7
5	2	1	7

360 min.

rep.	treat	control	elsewhere
1	2	2	6
2	3	3	4
3	3	0	7
4	0	6	4
5	3	0	7

Settling Preference

Species Thrips tabaci

Plant Cucumber "Korinda"

Treatment Tanacetum vulgare

Nr. individuals / unit 10

30 min

rep.	treat	control	elsewhere
1	2	6	2
2	3	4	3
3	5	3	2
4	8	0	2
5	3	6	1

60 min

rep.	treat	control	elsewhere
1	1	7	2
2	2	5	3
3	5	5	0
4	5	0	5
5	2	6	2

120 min.

rep.	treat	control	elsewhere
1	3	4	3
2	3	3	4
3	4	6	0
4	4	3	3
5	1	7	2

180 min.

rep.	treat	control	elsewhere
1	3	5	2
2	2	5	3
3	2	7	1
4	4	3	3
5	1	8	1

240 min.

rep.	treat	control	elsewhere
1	3	5	2
2	2	6	2
3	4	5	1
4	4	3	3
5	2	7	1

300 min.

rep.	treat	control	elsewhere
1	2	7	1
2	1	8	1
3	5	4	1
4	4	3	3
5	7	3	3

360 min.

rep.	treat	control	elsewhere
1	1	6	3
2	1	9	0
3	6	3	1
4	4	4	2
5	4	6	0

No choice assay: Feeding and Oviposition rate

Water-control		*A. absinthium*		Florissa		*T. vulgare*	
damaged area (mm²)	number of eggs	damaged area (mm²)	number of eggs	damaged area (mm²)	number of eggs	damaged area (mm²)	number of eggs
14,875	5	11	7	12,25	6	14,5	7
2,375	4	11	7	0,875	0	7,375	4
12,625	6	0	3	12,5	6	12,125	7
9,875	5	12,125	7	12,75	1	1,375	1
14,75	4	11	3	9,875	3	0	0
12,625	6	13,75	5	11,625	6	12,25	2
11,125	5	8,75	5	10,625	2	3,5	3
0	0	12,75	3	11,875	2	13,75	3
1,125	1	9	6	8,25	4	9,125	3
14	6	0,25	0	4,375	2	10,625	5
9,625	6	8,875	4	17,25	8	9,875	5
7,875	2	15	7	9,125	4	14,75	6
8,5	5	0,375	0	6,25	8	15,75	8
11,125	8	12,25	8	19,125	7	1,375	5
13	9	12	7	7,25	5	0	0
16,125	8	12,5	5	2,875	3	5,375	4
7,25	5	8,375	4	8,75	5	5	7
7,125	5	11,25	7	7,375	5	7,375	7
10	7	11,875	6	1	2	4,875	4
3,5	3	6,375	6	9,75	8	5,25	6
13,625	7	10,25	6	4,5	5	7,125	6
7,625	8	5,5	8	6	4	6,625	5
7,125	4	7,625	6	9	5	8,875	3
9,375	4	10,375	6	3	3	4,25	4
12,75	9	4,25	5	10,625	8	4,875	5
4	6	7,875	9	3,625	3	4,625	3
11,5	2	2,5	6	31	5	27	4
45,5	7	8,5	7			18,5	5
		10,75	5				

Thickness of upper epidermis cells in µm (always three measurements per one leaf)

Water		*A.absinthium*		Florissa		*T.vulgare*	
7,17	15,4	11,02	10,8	14,24	6,34	12,42	16,8
10,09	10,67	11,26	10,24	11,75	9,77	12,63	15,25
11,9	10,8	9,97	7,57	10,06	9,75	10,77	15,92
11,5	16,61	16,29	11,56	7,67	18,77	11,77	16,59
11,55	12,56	13,69	13,2	9,21	13,17	11,34	15,4
12,17	15,81	14,26	12,18	10	22,2	8,93	20,52
11,53	8,45	12,7	13,29	12,69	18,47	14,48	14,67
13,13	11,58	16,34	15,33	8,1	24,61	12,47	11,85
8,05	10,14	13,01	15,74	11,57	19,96	12,3	11,32
12,89	16,43	10,95	11,83	8,99	14	15,83	13,36
9,76	9,93	9,64	10,28	7,63	21,3	16,8	15,25
15,45	9,37	11,75	11,63	9	13,33	13,89	13,18
11,16	9,56	9,36	17,48	13,15	19,41	14,25	13,42
12,95	10,03	11,02	10,36	10,85	9,18	13,9	11,55
11,07	8,88	10,51	12,18	13,62	10,1	13,57	13,28
9,72	7,04	9,88	10,53	14,84	20,71	13,06	9,96
8,27	8,96	10,92	10,75	16,11	28,53	11,87	13,49
9,34	7,63	11,34	14,92	12,77	13,89	13,7	10,69
13,93	6,53	10,4	13,36	17,32	9,99	13,39	14,89
13,7	8,48	11,92	14,17	10,93	10,2	13,05	14,59
19,52	7,16	12,28	15,25	14,6	8,1	9,57	8,7
10,48	8,88	14,02	10,2	4,5	16,29	7,78	12,43
10,25	10,91	11,31	9,52	7,99		9,63	13,48
9,23	8,07	16,39	11,78	7,27	12,36	8,41	12,73
11,15	7,63	10,4	11,14	11,04	13,46	9,39	16,98
10,41	14,35	9,79	9,8	14,9	15,09	9,69	8,61
10,68	11,02	9,6	10,61	17,88	10,95	8,46	10,5
9,26	10,43	8,81	9,54	10,05	6,92	12,05	10,62
12,16	10,84	11,84	12,81	9,86	10,21	10,55	12,51
13,99	8,6	13,09	11,7	7,88	9,13	12,2	14,09
7,42	11,99	12,78	16,85	11,23	16,45	12,24	11,33
12,59	15,74	10,71	15,47	10,4	15,16	10,44	8,7
15,25	12,78	10,54	19,23	10,41	11,75	14	9,03
11,3	9,76	13,32	21,16	12,49	10,71	8,26	10,28
10,44	10,23	9,98	19,44	11,93	9,85	9,07	7,66
9	12,88	10,84	11,66	9,03	9,22	9,56	9,22
7,6	13,44	16,98	10,91	8,31	12,23	14,9	9,34
10,54	14,24	11,02	20,05	11,34	12,09	18,91	12,13
10,98	11,07	9,5	13,49	9,21	13,38	16,33	10,2
7,87		13,15		11,85		12,69	
7,9		8,72		11,5		13,68	
10,77		11,32		8,45		11,08	

Thickness of upper epidermis cell walls in µm

Water			*A. absinthium*			*T. vulgare*		Florissa		
Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 3
0,5	0,48	0,53	0,94	0,82	0,61	0,78	0,78	0,52	0,78	0,91
0,58	0,41	0,52	0,78	0,83	0,27	1,17	0,89	0,46	0,5	1,04
0,53	0,27	0,69	0,99	0,88	0,61	0,87	0,82	0,63	0,58	0,73
0,63	0,55	0,68	0,84	0,92	0,48	0,72	0,55	0,48	0,94	0,55
0,67	0,61	0,41	0,83	0,82	0,54	0,87	0,71	0,69	0,56	0,71
0,65	0,64	0,48	0,65	0,55	0,75	0,68	0,68	0,7	0,68	0,69
0,49	0,78	0,64	0,55	1,11	0,67	0,78	0,87	0,48	0,53	0,63
0,53	0,43	0,68	0,8	0,76	0,78	0,92	0,77	0,37	0,49	0,88
0,64	0,73	0,67	0,48	0,79	0,43	0,78	0,53	0,56	0,49	0,82
0,55	0,34	0,35	1,09	0,72	0,76	0,7	0,61	0,55	0,65	0,79
0,48	0,41	0,63	0,76	0,54	0,72	0,68	0,62	0,48	0,58	0,79
0,48	0,7	0,79	0,67	0,58	0,64	0,87	0,68	0,53	0,73	0,78
0,63	0,68	0,73	0,65	0,75	0,64	0,95	0,61	0,61		1,01
0,67	0,67	0,68	0,56	0,41	0,58	0,88	0,63	0,55		0,73
0,63	0,41	0,82	1,19	0,65	0,83	0,76	0,69	0,58		0,63
0,72	0,64		1,25	0,76	0,78	0,94	0,65	0,64		0,95
0,7	0,58		1,14	0,82	0,55	0,79	0,68	0,58		0,68
0,58			1,55	0,54	0,65	0,74	0,68	0,58		0,58
0,61			1,5	0,82	0,79	0,68	0,48	0,58		0,48
0,49			0,87	0,61	0,58	0,68	0,56	0,4		0,78
0,64			1,31	0,83	0,72	0,94	0,82	0,64		0,58
			1,1	0,55	0,58	0,99	0,87			0,52
			1,16	0,54	0,75		0,83			0,68
			1,14	0,76	0,89		0,54			0,71
				0,61	0,53		0,69			0,85
					0,67		0,55			0,75
							0,48			0,76
										0,87