Bioecological Characteristics and Biological Control of *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in Georgia

Natalia Kharabadze

The dissertation been submitted to the Council of Agrarian Sciences of the Agricultural University of Georgia to obtain the academic degree of Doctor of Agricultural Sciences

Scientific Supervisors:

- Medea, Burjanadze, **Professor Dr.;**
- Nona, Chkhaidze **Pofessor Dr.;**
- Michael, Rostas, **Professor Dr.;**

Agricultural University of Georgia

Tbilisi 2023

აზიური ფაროსანას *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) ბიოეკოლოგიური თავისებურებები და ბიოლოგიური კონტროლი საქართველოში

ნატალია ხარაბაძე

სადისერტაციო ნაშრომი წარდგენილია საქართველოს აგრარული უნივერსიტეტის აგრარულ მეცნიერებების საბჭოზე აგრარულ მეცნიერებათა დოქტორის აკადემიური ხარისხის მოსაპოვებლად

სამეცნიერო ხელმძღვანელები:

- მედეა ბურჯანაძე, დოქტორი, პროფესორი
- ნონა ჩხაიძე, დოქტორი, პროფესორი
- მაიკლ როსტასი, დოქტორი, პროფესორი

საქართველოს აგრარული უნივერსიტეტი

თბილისი 2023

საქართველოს აგრარული უნივერსიტეტი

სადოქტორო სკოლა

სამეცნიერო მიმართულების კომისიის რეკომენდაცია

დისერტანტი: ნატალია ხარაბაძე

დისერტაციის სათაური: „აზიური ფაროსანას *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) ბიოეკოლოგიური თავისებურებები და ბიოლოგიური კონტროლი საქართველოში," **"Bioecological Characteristics and Biological Control of *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in Georgia"**

დისერტაციის დაცვის თარიღი: 18 დეკემბერი,2023

სადისერტაციო კომისია:
რეცენზენტი 1: / მზია ბერუაშვილი/
რეცენზენტი 2: / მანანა კახაძე/
დაცის კომისიის თავმჯდომარე, /გიორგი ჯაფოშვილი/: _____
დაცვის კომისიის წევრი, / რუსუდან სხირტლაძე /_____
დაცვის კომისიის წევრი, /ზურაბ ხიდაშელი /_____
დაცვის კომისიის წევრი, / მედეა ბურჯანაძე /_____
დაცვის კომისიის წევრი, / ნონა ჩხაიძე /_____
დაცვის კომისიის წევრი, /მაიკლ როსტასი /_____

რეკომენდებულია დაცვისათვის სამეცნიერო მიმართულების კომისიის მიერ.

თავჯდომარე, /ცოტნე სამადაშვილი/ _____

წევრი, /დავით ბედოშვილი /: _____

წევრი, /თეო ურუშაძე/ : _____

წევრი, /ვალოდია ელისაშვილი /: _____

წევრი, /ია ფიფია /: _____

სადოქტორო პროგრამების კოორდინატორი: _____ / ნატო კობახიძე/

თარიღი:

ანოტაცია

წარმოდგენილი სამუშაო ეხება სასოფლო სამეურნეო კულტურების ერთ-ერთი მნიშვნელოვანი მავნე მწერის აზიური ფაროსანას - *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) შესწავლას, მის ბიოეკოლოგიური მახასიათებლების დადგენას, ადგილობრივი ბუნებრივი მტრების გამოვლენას, მის წინააღმდეგ ეფექტიანი და გარემოსათვის უსაფრთხო ღონისძიებების შემუშავებას.

კვლევის აქტუალურობა - სასოფლო სამეურნეო კულტურების ერთ-ერთი უმთავრესი მავნე პოლიფაგი მწერი-აზიური ფაროსანა *H. halys* საქართველოში აღინიშნება 2015 წლიდან. საკვების სიუხვემ, კლიმატურმა პირობებმა, მწერის მაღალმა რეპროდუქტიულობამ, ბუნებრივი მტრების სიმცირემ, ხელი შეუწყო მწერის სწრაფ ადაპტაციას და გამრავლებას საქართველოში. დღესდღეობით საგანგაშო მდგომარეობაა, რადგან მწერის გავრცელებამ და აგრესიულმა ქმედებამ დასავლეთ საქართველოში დიდი ზიანი მიაყენა თხილის ბაღებს და გამოიწვია მნიშვნელოვანი ეკონომიკური ზარალი (საქართველოს გარემოს დაცვისა და სოფლის მეურნეობის სამინისტრო; Kharabadze et al. 2019). ამიტომ, აზიური ფაროსანას წინააღმდეგ ბრძოლის კომპლექსურ ღონისძიებათა სისტემის დამუშავება და მათი რიცხოვნობის რეგულირება, მეტად აქტუალურია. ამ ღონისძიებათა ერთობლიობაში კი მნიშვნელოვანი ადგილი უკავია ადამიანისა და გარემოსათვის უსაფრთხო ბიოლოგიური მეთოდების შემუშავებას, ბუნებრივი მტრების გამოვლენას და მათ გამოყენებას მავნებლების ინტეგრირებული მართვის სისტემაში (IPM).

აზიური ფაროსანას წინააღმდეგ ბრძოლისათვის საქართველოში, ისევე როგორც მრავალ ქვეყანაში, ძირითადად გამოიყენება ქიმიური ინსექტიციდები, რომლებიც ანადგურებენ სასარგებლო მწერებსაც, აბინძურებენ გარემოს და არღვევენ ეკოლოგიურ ბალანსს.

ქიმიური პესტიციდების გამოყენების შემცირების მიზნით, აზიური ფაროსანას წინააღმდეგ მთელს მსოფლიოში მიმდინარეობს კვლევები, ეკოლოგიურად უსაფრთხო, ბიოლოგიური საშუალებების შემუშავებისათვის, რომლებიც მოიცავს ბუნებრივი მტრების - მტაცებლების, პარაზიტოიდების ენტომოპათოგენური

მიკროორგანიზმების (ვირუსები, პროტოზოები, სოკოები, ნემატოდები) ძიებასა და გამოვლენას (Abram et al. 2017; Formella et al. 2019; Andreadis et al. 2021; Francati et al. 2021; Japoshvili at al., 2022), ასევე ბიოტექნიკური საშუალებების, როგორიცაა ფერომონების შექმნას. ასეთი მიდგომა აამაღლებს მავნებელთან ბრძოლის ეფექტიანობას, იგი უსაფრთხო იქნება ადამიანისა და გარემოსათვის, ზიანს არ მიაყენებს ბიომრავალფეროვნებას, შეინარჩუნებს ეკოლოგიურ ბალანს და უპასუხებს გაეროს განვითარების 2015-2030 წლის მიზნებს.

კვლევის სიახლე - საქართველოს პირობებში პირველად იქნა შესწავლილი აზიური ფაროსანას ბიოეკოლოგიური თავისებურებები: ა) თაობათა რაოდენობა, ბ) მკვებავი მცენარეები, გ) სქესობრივი ინდექსი, რომლებიც მნიშვნელოვანია შემდგომში მათ წინააღმდეგ ბრძოლის ღონისძიებათა ეფექტიანად განხორციელებისათვის. თხილის კულტურის მაგალითზე გამოვლენილია მკვებავი მცენარის გამძლეობის მარკერები. ასევე, გამოვლენილი და შეწავლილია მისი ადგილობრივი ბუნებრივი მტრები (მტაცებლები, ენტომოპათოგენური სოკოები), რომლებსაც აქვთ პოტენციალი წარმატებით იყვნენ გამოყენებული, როგორც აზიური ფაროსანას ბიოლოგიური კონტროლის აგენტები.

წარმოდგენილი ნაშრომის მიზნია - აზიური ფაროსანას ბიოეკოლოგიური მახასიათებლების შესწავლა, ბუნებრივი მტრების გამოვლენა, ბიოლოგიური კონტროლში გამოყენების მიზნით.

დასახული მიზნების მიღწევისათვის შესრულდა შემდეგი ამოცანები:

- აზიური ფაროსანას ბიოეკოლოგიური თავისებურებების დადგენა
- მწერისა და მკვებავი მცენარის ურთიერთდამოკიდებულებისა და გამძლეობის მარკერების გამოვლენა
- მწერის ადგილობრივი ბუნებრივი მტრების გამოვლენა
- გამოვლენილი ბუნებრივი მტრების ეფექტიანობის დადგენა

სამეცნიერო ლიტერატურის მიმოხილვა - აზიური ფაროსანა - *Halyomorpha halys* - ფაროსანი ბაღლინჯოების (Pentatomidae) ოჯახის წარმომადგენელი მავნე, ინვაზიური მწერია (Lee et al., 2013a). ისევე როგორც Pentatomidae-ს ოჯახის მრავალ მავნე სახეობა, აზიურ ფაროსანაც მაღალი პოლიფაგია და გავრცელებულია ყველა კონტინენტის ჩრდილოეთ ნახევარსფეროს ქვეყნებში. აღწერილია მისი 300-ზე მეტი მასპინძელ მცენარე (Venugopal et al., 2014, Ciceoi et al., 2017). ევროპაში მისი გავრცელების შესახებ 2013 წლამდე ცნობები არ მოიპოვებოდა, თუმცა 2014 წლის შემდეგ მოხდა მისი ინტენსიური გავრცელება. დღეისათვის იგი ევროპის 15 ქვეყანაშია გავრცელებული (Ciceoi et al., 2017). მწერი თავისი განვითარების სხვადასხვა ფაზაში, კვებით აზიანებს სასოფლო სამეურნეო, დეკორატიულ და ველურ მცენარეებს რითაც საფრთხეს უქმნის ბიომრავალფეროვნებას (Peiffer and Felton, 2014, Acebes, 2016). მწერს ახასიათებს გადარჩენის და გამრავლების მაღალი მაჩვენებელი. სხვადასხვა კლიმატურ და გეოგრაფიულ პირობებში იძლევა 1-6 თაობას სავეგეტაციო პერიოდის განმავლობაში. გარდა ამისა, *H. halys* გამოსაზამთრებლად მიგრირებს შენობებში, სახლებში, სადც რჩება მთელი ზამთრის განმავლობაში. მწერი გამოყოფს - მყრალ სუნს, რაც ქმნის სოციალურ და ჯანმრთელობის პრობლემებს (იწვევს ალერგიას) მოსახლეობაში (Hoebeke and Carter, 2003).

H. halys-ს ბუნებრივი გავრცელების არეალია ჩინეთი, იაპონია და კორეაა (Hoebeke and Carter, 2003). დღეისათვის ის უმნიშვნელოვანეს პრობლემას წარმოადგენს, როგორც ამერიკის, ასევე ევროპის მრავალი ქვეყნის სასოფლო სამეურნეო სექტორისათვის, მათ შორის საქართველოსათვისაც. დასავლეთ საქართველოში (აფხაზეთში) ის შემოიჭრა 2012 წელს სოჭის ოლიმპიადის შემდეგ (Gapon, 2016) და 2016 წლისათვის მიაღწია აჭარამდე (Meskhi, 2017) და ამ დროის განმავლობაში, მის მიერ გამოწვეული ზარალი 54 მლნ. დოლარს შეადგენდა (Ministry of Finance of Georgia, 2017). *H. halys* იტალიასა და საქართველს პირობებში ყველაზე მეტად აზიანებს თხილს (Bosco et al., 2018,). არსებული მონაცემების საფუძველზე მწერის საზიანო მოქმედება საჭიროებს მავნე მწერთა ინტეგრირებული მართვის ღონისძიებებს (Bosco et al., 2018).

გარემოს მზარდი დაბინძურების და სოფლის მეურნეობის ინტენსიური განვითარების პირობებში, განსაკუთრებით დიდი მნიშვნელობა ენიჭება მავნე მწერებისაგან მცენარეთა დაცვის ინტეგრირებული სისტემის შემუშავებას. ამ

სისტემაში ქიმიური დაცვის საშუალებების გამოყენება დასაშვებია მხოლოდ იმ შემთხვევისათვის, როდესაც მავნე მწერების რიცხოვნობა აღემატება მავნეობის ეკონომიკურ ზღვარს; ამ საქმიანობაში უპირატესობა ენიჭება მავნე ორგანიზმების წინააღმდეგ ბრძოლის ბიოლოგიურ მეთოდს, მცენარის იმუნიტეტის გაზრდას, გამძლე ჯიშების სელექციას. მავნებლის წინააღმდეგ ინტეგრირებული დაცვის სისტემის შემუშავება შეუძლებელია მისი ბიო-ეკოლოგიური თავისებურებების საფუძვლიანი ცოდნის გარეშე.

H. halys რიცხოვნობის ეკოლოგიურად უსაფრთხო მეთოდებით რეგულირებისათვის აუცილებელია მისი ბიოლოგიური თავისებურებების, მკვებავი მცენარეების და ბუნებრივი მტრების შესწავლა.

კვლევის მეთოდები

კვლევის ობიექტი - იყო აზიური ფაროსანა - *H. halys,* მისი მკვებვი მცენარეები, ბუნებრივი მტრები - ჩოქელა (Mantis species), აგრეთვე აზიური ფაროსანას პოპულაციებიდან გამოყოფილი ენტომოპათოგენური სოკოს შტამები (*Beauveria bassiana*, *Isaria fumosorosea*).

კვლევის მეთოდები

კვლევები განხორციელდა 2018-2021 წლებში,მარტიდან ოქტომბრის შუა რიცხვებამდე, დასავლეთ საქართველოს სამ მუნიციპალიტეტში: წინასწარ შერჩეულ საცდელ ნაკვეთებზე. იმერეთში საცდელი ნაკვეთი მდებარეობდა ვანში (42°05'39.9"N 42°30'12.5"E), ამ საკარმიდამო ნაკვეთზე გაშენებული იყო ხილის შერეული ტიპის ბაღი, (ვაშლი, მსხალი, ბროწეული, თხილი, ყურძენი, ტყემალი, ქლიავი, ბალი) და ბოსტნეული კულტურები. ამ ნაკვეთს ყველა მხრიდან ესაზღვრებოდა სხვა საკარმიდამო ნაკვეთები, ნაკვეთის ფართი იყო 0.5 ჰა. გურიაში საცდელი ნაკვეთი შეირჩა სოფელი ნინოშვილში (42°02'24.8"N 41°57'21.9"E) რომელიც მოიცავდა როგორც

საკარმიდამო ასე სასოფლო სამეურნეო სავარგულსა და ტყეს. ამ ნაკვეთის ყველაზე დაბალი წერტილი ზღვის დონიდან 73 მეტრზეა, ხოლო ყველაზე მაღალი 200 მეტრზე. მოცემულ ნაკვეთს ესაღვრება ტყე და საკარმიდამო ნაკვეთები, რომლებზეც ძირითადად გაშენებულია თხილი ნაკვეთი გაყოფილი იყო ორ ნაწილად და საერთო ფართი შეადგენს 15 ჰექტარს. სამეგრელოში საცდელი ნაკვეთი იყო სოფელ დარჩელიში (42°25'14.4"N 41°39'17.6"E), ნაკვეთის ჩრდილო-დასავლეთით მდებარეობს მდინარე პატარა ენგური, სამხრეთით - დაუმუშავებელი ტერიტორია, ხოლო აღმოსავლეთით თხილის ბაღები, ნაკვეთი მოიცავს 1 ჰექტარს. მკვებავ მცენარეთა გამოსავლენად ექსპედიციები ხორციელდებოდა სამკვირიანი ინტერვალებით. დაკვირვებები მიმდინარეობდა წინასწარ, შემთხვევითობის პრინციპით შერჩეულ ხეებზე.

აზიური ფაროსანას თაობათა რაოდენობის დადგენის მიზნით, შეგროვდა მონაცემები მისი განვითარებისათვის საჭირო ტემპერატურის, ტენიანობის, დღის ხანგრძლობის კრიტიკული ზღვრების შესახებ და შედარდა დასავლეთ საქართველოს კლიმატურ პირობებს. ასევე აღრიცხული იყო კვერცხდებების თარიღები და მოხდა შედარება მოსაზღვრე რეგიონში სხვა მეცნიერების მიერ ჩატარებულ კვლევებთან.

მკვებავ მცენარეთა გამძლეობის მარკერებს (ფენოლოგია, ლიგნინის შემცველობა) ვიკვლევდით თხილის ორი ჯიშის, ბერძნულასა (=ბერძნული, *Corylus avellana* L.) და დედოფლის თითის (=აკაკი წერეთლის თითი, ბადემი, *C. colchica* Albov.) მაგალითზე, მცენარეები იმყოფებოდნენ სრულად მსხმოიარობის ფაზაში (ხომასურიძე, 1978; სიჭინავა, 2005; მიროტაძე, 2011).

თხილის დაზიანების ხარისხი შეფასდა და დაზიანების კატეგორიები დადგინდა ჰედსტრომის მეთოდის მიხედვით (Hedstrom et al., 2014).

ჩატარდა მკვებავ მცენარეთა მორფოლოგიური კვლევები, სადაც თხილის ნაყოფის ნაჭუჭის სისქე გაზომილი იყო ტექნიკური სიმწიფის ფაზაში შტანგენფარგალით (სიჭინავა, 2005). ფორმირების პროცესში მყოფი ნაჭუჭის და მისი სტრუქტურული ელემენტების სისქე განისაზღვრა სინათლის მიკროსკოპის (МБР-1) ოკულარული ხრახნიანი მიკრომეტრით (MOB-1-15X OMO USSR) სხვადასხვა გადიდებაზე.

ანატომიური კვლევისათვის მცენარის ანათლები დამზადდა მიკროტომის (Microtome Leitz Germany 6619) მეშვეობით, შეიღება საფრანინით (1:1000 გ/მლ საფრანინი/დისტილირებული H_2O) 24 სთ განმავლობაში. ანათლები შესწავლილი იქნა სინათლის მიკროსკოპით (МБР-1).

ფაროსანას და თხილის ფენოლოგური ფაზების შესწავლა ჩატარდა მიღებული მეთოდიკების მიხედვით. თხილზე აღირიცხებოდა ნაყოფის გამონასკვის, რძისებრი და ტექნიკური სიმწიფის ფაზები (სიჭინავა, 2005). ფაროსანას შემთხვევაში: მეზამთრეობაში გადასვლა, მეზამთრეობიდან გამოსვლა, კვერცხდება, მატლისა და იმაგოს ფაზები, ვოლტინობა დადგინდა ერიკ ბერგმანის მეთოდის მიხედვით (Bergmann et al., 2015).

აზიური ფაროსანას ბუნებრივი მტრების მოპოვება განხორციელდა საქართველოს სხვადასხვა, ჩვენს მიერ ყველა გამოკვლეულ რეგიონში. იდენტიფიცირებას ვახდენდით მწერების სარკვევის გამოყენებით (ნებიერიძე, სავენკო, 1957).

აზიური ფაროსანას - *H. halys* პოპულაციებში ერთეული მწერებიდან იზოლირებულია ენტომოპათოგენური სოკოები, და მოვახდინეთ მათი კულტივირება ხელოვნურ საკვებ არეებზე, 23 ± 2° C- ზე 12-15 დღის განმავლობაში (Humber, 1997; Burjanadze et al., 2020) ლაბორატორიაში.

იზოლირებული მონოკულტურების მორფოლოგიური და მოლეკულური შესწავლა განხორციელდა მწერების პათოლოგიაში არსებული მეთოდებით (Evlakhova, 1974; Roberts, 1992; Humber, 1997; Vellinga, 1988; Brandfass and Karlovsky, 2008; Sambrook, Russell, 2001; Toju et al., 2012; White et al., 1990). ასევე შესწავლილი იყო ამ იზოლატების შემდეგი ფერმენტული (ქიტინაზური, პროტეაზული და ლიპაზური) აქტიურობა (Nahar et al., 2013; Vyas and Deshpande,1989).

ენტომოპათოგენური სოკოების ბიოლოგიური ეფექტიანობის დადგენისათთვის, საველე და ლაბორატორიული ცდებისათვის, სუსპენზია მზადდებოდა 2 კვირიანი სოკოვანი კულტურებისგან, რომლებიც იზრდებოდნენ კარტოფილის დექსტროზა აგარზე (PDA), 23 ± 2° C-ზე, რომელთაც აღენიშნებოდათ კარგი სპორულაცია. სუსპენზიაში გამოყენებული იყო გამოხდილი წყალი, რომელიც შეიცავდა სპორების დამაცალკავებელს - Twin 80 0.01%. ხსნარში სპორების

კონცენტრაცია გაიზომა ჰემოციტომეტრის საშუალებით. ცდებში გამოყენებული იყო სამი კონცენტრაცია $1x10^6$, $1x10^7$ და $1x10^8$ (Humber 1997; Lacey & Kaya, 2007; Kunelauri et al., 2019).

მიღებული შედეგების სტატისტიკური ანალიზისთვის გამოვიყენეთ შემდეგი ტესტები: T-ტესტი თხილის სხვადასხვა ჯიშებში ნაჭუჭის სისქეებს შორის სხვაობის დასადგენად; F-ტესტი ბერძნულისა და დედოფლის თითის ნაჭუჭის სისქის ცვალებადობის დასადგენად.

ჩოქელას (Mantodea) მორფოლოგიური ნომენკლატურის დასადგენად და მისი ინდივიდების მორფომეტრულ მონაცემებს შორის ძირითადი სხვაობის დასადგენად გამოყენებული იქნა ANOVA. ჩოქელას კვების ინტენსივობა შემოწმდა სტატიკურად დღეების და საათების მიხედვით კაპლან-მეიერის მეთოდის გამოყენებით. გამოთვლები განხორციელდა IBM SPSS Statistics 23-ზე (IBM Corp. Released 2015)

ლაბორატორიაში გამოცდილი პრეპარატების (ენტომოპათოგენური სოკოებისა და ნემატოდების შტამები, ასევე საკონტროლოდ გამოყენებული ქიმიური ინსექტიციდის) მიერ გამოწვეული სიკვდილიანობის ყველა მონაცემი შესწორდა Abbott-ის ფორმულის (Abbott, 1925) გამოყენებით, ხოლო საველე სამუშაოებისას სიკვდილიანობას აღვრიცხავდით Franz-ის ფორმულით (Franz, 1968). ლარვების სიკვდილიანობის პროცენტი თითოეული კონცენტრაციისთვის გაანალიზებული იყო Onaway ANOVA-ს გამოყენებით; საშუალო მაჩვენებლების გამოყოფა მოხდა Tukiye's საშუალოების გამოყოფის ტესტით. $P<0.01$ შემთხვევაში სიკვდილიანობა ჩათვლილი იყო სტატისტიკურად მნიშვნელოვნად. კაპლან-მეიერის ანალიზის მეთოდი გამოიყენებოდა როგორც საშუალო სიკვდილიანობის, ასევე მედიანური ლეტალური დროის (LT50) დასადგენად (GraphPad Prism 9.1). დოზებისა და ექსპოზიციის დროს შორის მნიშვნელოვანი განსხვავებების გამოსათვლელად, განხორციელდა Onaway ANOVA, SPSS 23.0 პროგრამული პაკეტის გამოყენებით $P<0.01$ და $P<0.05$ დონეზე.

კვლევის მნიშვნელოვანი შედეგები:

აზიური ფაროსანა, მისი მკვებავი მცენარეები და ბუნებრივი მტრები შეგროვდა დასავლეთ საქართველოში (გურია, სამეგრელო, აჭარა) და აღმოსავლეთ საქარველოში (თბილისის შემოგარენი).

დასავლეთ საქართველოს სუბტროპიკულ რაიონებში *H. halys*-ს ვითარდება ორი ან სამი თაობა. ჩვენს პრობებში დიაპაუზიდან გამოსვლა იწყება აპრილის შუა რიცხვებიდან და დიაპაუზაში გადადის ოქტომბრის შუა რიცხვებში. ეს მაჩვენებლები მერყეობს კლიმატური პირობების მიხედვით.

H. halys გამოირჩევა მაღალი ნაყოფიერებით, მის ერთ კვერცხნადებში საშუალოდ არის 28 კვერცხი.

წლების მიხედვით იცვლებოდა მწერის სქესთა თანაფარდობა, მაგრამ ეს თანაფარდობა ყოველთვის იყო მდედრების სასარგებლოდ, რომელიც განსაკუთრებით მაღალი იყო 2021 წელს. კვერცხების გამოჩეკვა 100-78%-ის ფარგლებში მერყეობდა.

აზიური ფაროსანას პოლილფაგური ბუნება შეინიშნებოდა ექსპერიმენტებშიც. მისი კვება დაფიქსირდა 38 სახეობის მცენარეზე, რომლებიც მოიცავს 31 გვარსა და 23 ოჯახს. მკვებავი მცენარეების სამი სახეობა *Agave americana* L. (Asparagaceae) , *Sambucus nigra* L.(Adoxaceae) , *Laurus nobilis* L. (Lauraceae) პირველად ჩვენს მიერ გამოვლინდა დასავლეთ საქართველოს პირობებში.

მწერის აზიანებს მრავალ სასაოფლო სამეურნე კულტურას, დეკორატიულ და ტყის სახეობებს, თუმცა საქართველოს პირობებში მწერის უპირატესობას ანიჭებს თხილს და სიმინდს.

სავეგეტაციო პერიოდის განმავლობაში, იცვლება მწერის მკვებავი მცენარეების სახეობები. მაისიდან ივნისის ბოლომდე *H. halys* უპირატესობას ანიჭებს თხილს. ივლისიდან აგვისტოს ბოლომდე ის იკვებება ბოსტნეულსა და სიმინდზე. ხოლო აგვისტოდან ოქტომბრამდე - ხეხილოვან მცენარეთა ნაყოფებზე და ველურ ბალახოვან მცენარეებზე.

ჩვენი კვლევის ერთ-ერთი ამოცანა იყო შეგვესწავლა თხილის, როგორც საქართველოს სოფლის მეურნეობის სტრატეგიული კულტურის დაზიანება და მისი გამძლეობა აზიური ფაროსანას მიმართ. გამორიკვა რომ, თხილი ზიანდება ყველა

ასაკის *H. halys*-ს მიერ. ყველაზე მეტი კვერცხდება აღინიშნა თხილზე, რაც მიანიშნებს იმაზე, რომ თხილი ყველაზე მეტად მისაღები მკვებავი მცენარეა *H. halys*-სთვის ჩვენს პირობებში. მწერი კვერცხს დებს სადედე ტოტზე განთავსებულ, წვეროდან მე-2 ან მე-3 ფოთოლზე. ახლადგამოჩეკილი ფაროსანას ნიმფები 2 დღის შემდეგ იწყებენ აქტიურ კვებას თხილის ფოთლებიდან და ახლადგამონასკვული ნაყოფებიდან. კვებითი დაზიანება კარგადაა გამოხატული ნაყოფებზე და ფოთლებზე და ნაკლებად ფოთლებზე. ნაყოფის დაზიანების ხარისხი დამოკიდებულია *H. halys*-ის ასაკსა და თაობაზე, თუმცა მისი ხორთუმის სიგრძე ყოველთვის მეტია ვიდრე თხილის ნაჭუჭის სისქე, რაც საშუალებას აძლევს მწერს, რომ ყველა ასაკში დააზიანოს თხილის ნაყოფი. მწერის ნერწყვი შეიცავს სხვადასხვა ფერმენტებს, რომლებიც აუცილებელია საჭმლის მონელებისათვის, მაგრამ მასში არ არის ლიგნინის დამშლელი ფერმენტები, ამიტომ, როგორცკი თხილის ნაჭუჭში დაიწყება ლიგნიფიკაციის პროცესი თხილის ნაყოფი *H. halys* -ს კვებისათვის მიუღებელი ხდება. ნაჭუჭის ლიგნიფიკაცია თხილის ჯიშებში სხვადასხვა დროს იწყება და იცვლება მეტეოროლოგიური პირობების მიხედვით. ლიგნინის წარმოქმნის უნარი გენეტიკურადაა განპირობებული და იცვლება ჯიშების მიხედვით. გამძლე ჯიშ ბერძნულას ნაყოფის ნაჭუჭში ლიგნიფიკაცია იწყება ადრე ვიდრე ჯიშ დედოფლის თითში. ლიგნიფიკაციის დამთავრება ჩვენს პირობებში ემთხვევა *H. halys*-ს პირველი თაობიდან მეორეში გადასვლის პერიოდს. ადრე მომწიფებულ თხილის ნაყოფებში ლიგნინის მაღალი შემცველობა განაპირობებს მწერის გადასვლას სხვა სახეობებზე. ჩვენს მიერ გამოვლენილი ეს ფაქტი არის მიზეზი იმისა თუ რატომ ამჟღავნებს ბერძნულა მეტ გამძლეობას ვიდრე დედოფლის თითი ერთსა და იმავე პირობებში. სხვაობა ამ ორი ჯიშის დაზიანების ხარისხს შორის მერყეობს 15-35%-ის ფარგლებში წლების მიხედვით.

აზიური ფაროსანას პოპულაციებიდან გამოვავლინეთ შემდეგი ენტომოფაგები - *Rhynocoris iracundus* (Roda, 1761)(Hemiptera: Reduviidae)*, Hierodula transcaucasica* Brunner von Wattenwyl, 1878 (Mantodea: Mantidae),*Iris polistictica* (Fischer-Walheim, 1846)(Mantodea: Eremiaphilidae)*, Dermeste* sp. (Coleoptera: Dermestidae), აქედან დეტალურად შევისწავლეთ *Hierodula transcaucasica.* ლაბორატორიული ექსპერიმენტის შედეგად დავადგინეთ რომ ზრდასრული *Hierodula transcaucasica* ერთ დღეში საშუალოდ სამ *H. halys*-ს იმაგოს ანადგურებს, ხოლო მის ნიმფას ერთ საათში

შეუძლია სამი აზიური ფაროსანას I-II ასაკის მატლების განადგურება. *Hierodula transcaucasica*-ს ასეთი ხარბი მტაცებლობა, საშუალებას გვაძლევს რომ ის გამოვიყენოთ *H. halys*-ს ბიოლოგიური კონტროლისათვის.

მსოფლიოში პირველად *H. halys* პოპულაციებიდან გამოვყავით შემდეგი ენტომოპათოგენური სოკოები *Beauveria bassiana, Isaria fumosorosea* შტამები. მათი სახეობრივი კუთვნილება დავადგინეთ მორფოლოგიური შესწავლის საფუძველზე. ფერმენტული ანალიზით დადასტურდა, რომ ჩვენს მიერ გამოყოფილ შტამებს (*Beauveria bassiana* - MB101; MB102; MB104; *Isaria fumosorosea* - MB 103) აქვთ მაღალი ქიტინაზური, პროტეაზული და ლიპაზური აქტიურობა, რაც აუცილებელია მწერის ინფიცირებისათვის. აღნიშნული შტამები, სხვა შტამებთან (*Beauveria bassiana* - MB 082; Isaria sp. - MB 011, Metarhizium sp. - MB 077, ARSEF 8318 -*Beauveria bassiana*, ARSEF 8319 – Metarhizium sp.) და ქიმიურ პრეპარატთან Bi-58 new (აქტიური ინგრედიენტი - დიმეთიოატი) ერთად გამოვცადეთ ლაბორატორიასა და ასევე საველე პირობებში. ყველაზე მაღალი ფერმენტული აქტიურობით და ბიოლოგიური ეფექტიანობით გამოირჩეოდა შტამი MB 101, რომელიც გამოყოფილი იყო *H. halys*-ის პოპულაციიდან.

შევისწავლეთ ერთ-ერთი პირველი ქართული მიკოპესტიციდის Bover-Ge-ს ეფექტიანობა *H. halys*-ს I-II ასაკის ნიმფებზე, ზრდასრულებსა და მოზამთრე ფაზებზე. დადგენილია, რომ Bover-Ge-ს ახასიათებს *H. halys*-ს მიმართ მაღალი სიკვდილიანობის გამოწვევის უნარი 65-90.5%.

ენტომოპათოგენური ნემატოდების გამოყენება მავნე მწერების ბიოლოგიური კონტროლისათვის ერთ-ერთი ძირითადი მიმართულებაა მსოფლიო მასშტაბით. შევისწავლილია. ადგილობრივ და იტალიის კოლექციებში არსებული ენტომოპათოგენური ნემატოდების შტამების ეფექტიანობა *H. halys*-ს მიმართ. კვლევაში გამოყენებული იყო შემდეგი შტამები: ქართული -*Heterorhabditis bacteriophora* (GEO), *Steinernema borjomiense* (GEO), იტალიური -*Heterorhabditis bacteriophora* (IT) და *Steinernema apuliae* (IT). დადგინდა რომ იტალიურ შტამებს ახასიათებს მაღალი ბიოლოგიური ეფექტიანობა აზიური ფაროსანას მიმართ ლაბორატორიაში და შესაძლებელია წარმატებით გამოვიყენოთ *H. halys*-ს ბიოლოგიურ კონტროლში.

დასკვნები

- დასავლეთ საქართველოს პირობებში აზიური ფაროსანა *H. halys*-ს შეუძლია ორი ან იშვიათად სამი თაობის განვითარება;
- პირველად იქნა ნაჩვენები, რომ შესწავლილ რეგიონებში გამოზამთრებული და I –II თაობის მწერები კვერცხდებისათვის უპირატესობას ანიჭებს თხილს. სხვადასხვა მცენარეებიდან მოპოვებულ ნადებებში კვერცხების რაოდენობა, ასევე გამოჩეკვის % მერყეობდა რეგიონისა და წლების მიხედვით და შეადგენდა 75 - 79 %-ს.
- აზიური ფაროსანას მოზამთრე პოპულაციებში მდედრების რაოდენობა 6-60%-ით აჭარბებდა მამრებისას 2018-2021 წლებში.
- პირველად ჩვენს მიერ, დასავლეთ საქართველოს პირობებში, აზიური ფაროსანას *H. halys* კვება დაფიქსირდა 38 სახეობის მცენარეზე დასავლეთ საქართველოს პირობებში, რომელთაგან *Agave americana, Sambucus nigra, Laurus nobilis* არ იყო შეტანილი მონაცემთა საერთაშორისო ბაზებში (EPPO, CABI).
- პირველად დადგენილია აზიური ფაროსანას მიმართ შედარებით გამძლე მკვებავი მცენარის გენოტიპის არსებობა. კერძოდ, თხილის ნაყოფის გამძლეობა დამოკიდებულია ნაჭუჭის ლიგნიფიკაციის დაწყების დროსა და ხარისხზე, აგრეთვე ლიგნინის წარმოქმნისა და მწერის კვებითი მოთხოვნების თანხვედრაზე. ზემოთქმულის გათვალისწინებით I და II თაობების ყველა ასაკის მწერები მეტად აზიანებს ჯიშს „დედოფლის თითი“, ვიდრე - „ბერძნულა“-ს.
- პირველად ჩვენს მიერ აზიური ფაროსანას პოპულაციებიდან გამოვლენილია შემდეგი ადგილობრივი ბუნებრივი მტრები: მტაცებლები - *Rhynocoris iracundus, Hierodula transcaucasica, Iris polistictica*, Dermeste sp., ხოლო ენტომოპათოგენური მიკროორგანიზმებიდან - სოკო *Beauveria bassiana, Isaria fumosorosea* შტამები.
- პირველად ჩვენს მიერ დადგინდა აზიური ფაროსანას ბუნებრივი მტრის ჩოქელას *Hierodula transcaucasica*-ს მტაცებლობის ხარისხი. ჩოქელას ზრდასრული ინდივიდი დღეში საშუალოდ სამ ზრდასრულ აზიურ

ფაროსანას, ხოლო მისი ნიმფა ერთ საათში ანადგურებდა სამ აზიური ფაროსანას ნიმფას.

- მორფოლოგიური და მოლეკულური კვლევების საფუძველზე დადგინდა აზიური ფაროსანას ადგილობრივი პოპულაციებიდან გამოყოფილი ენტომოპათოგენური სოკოების სახეობრივი შედგენილობა - *Beauveria bassiana* და *Isaria fumosorosea* შტამები.

- შესწავლილი იქნა იზოლირებული შტამების ფერმენტული (ქიტინაზური, ლიპაზური და პროტეაზული) აქტიურობა და დადგინდა რომ ყველაზე მაღალი ფერმენტული ქტიურობით ხასიათდებოდა შტამი MB 101 – 0.6057 ე/მლ, 1.564 ე/მლ, 1.06 ე/მლ შესაბამისად.
- იზოლირებული შტამების MB 101, MB 102,MB 103, MB 104 ეფექტიანობა აზიური ფაროსანას ნიმფებისა და ზრდასრულების მიმართ მერყეობს 60 – 90%-ის ფარგლებში.
- აზიური ფაროსანას ნიმფების წინააღმდეგ *Beauveria bassiana* - MB 101, MB 102, MB 104 გამოცდილი იყო სამი კონცენტრაციით $1x10^6$, $1x10^7$, $1x10^8$, სადაც სიკვდილიანობა შეადგენდა 80-99%,85-90%,70-90% შესაბამისად, ხოლო იმაგოს მიმართ 80-99%, 75-89%, 60-80% შესაბამისად.
- ბიოლოგიური ეფექტურობა აზიური ფაროსანას ნიმფების მიმართ *Isaria fumosorosea* - MB 103 შემთხვევაში ვარირებს 68-88%, ხოლო იმაგოს მიმართ შესაბამისად 70-90% შეადგენდა.
- აზიური ფაროსანას იმაგოს მიმართ გამოცდილი Bover-Ge-ს ბიოლოგიური ეფექტიანობა 72.0-90.5%-ია, ხოლო მეზამთრეობის ფაზაში - 65%-ს შეადგენდა.
- ენტომოპათოგენური ნემატოდების *Heterorhabditis bacteriophora* (GEO), *Steinernema borjomiense* (GEO), *Heterorhabditis bacteriophora* (IT) და *Steinernema apuliae* (IT) ბიოლოგიურმა ეფექტიანობამ აზიური ფაროსანას წინააღმდეგ მიაღწია 53.3% , 40%, 95.3%, 93.3% შესაბამისად.

რეკომენდაციები

მცენარეთა დაცვის, გარემოს დაცვის და სოციალურ-ეკონომიკური სფეროს მოთხოვნების გათვალისწინებით შედგენილია შემდეგი რეკომენდაციები:

1. თხილის ბაღების გაშენებისას უპირატესობა მიენიჭოს ჩვენს მიერ გამოვლენილ, შედარებით გამძლე ჯიშს ბერძნულს.
2. აზიური ფაროსანას წინააღმდეგ ბრძოლისათვის ფართოდ იყოს გამოყენებული ჩვენს მიერ დადგენილი მისი ბუნებრივი მტრები - მტაცებელი მწერი ჩოქელა და ენტომოპათოგენური სოკოები.
3. საქართველოს გარემოს დაცვისა სოფლის მეურნეობის სამინისტროსა და საგანმანათლებლო დაწესებულებების მიერ დაიწყოს ფართომასშტაბიანი ღონისძიებები აზიური ფაროსანას ჩვენს მიერ დადგენილი ბუნებრივი მტრების დაცვის, მოსახლეობისადმი გაცნობის და გამოყენების მიმართულებით.
4. გამრავლდეს და მოსახლეობაში გავრცელდეს ჩვენს მიერ გამოცემული საინფორმაციო ფურცელი ,,ჩოქელა - აზიური ფაროსანას ბუნებრივი მტერი“
5. გამოიცეს და გავრცელდეს ინფორმაცია ადგილობრივი ენტომოპათოგენური სოკოების საფუძველზე დამზადებული პრეპარატების შესახებ.

დისერტაციის შემდგომი განვითარებისა და გამოყენების პერსპექტივა:

მიუხედავად მსოფლიო მასშტაბით ჩატარებული და მიმდინარე კვლევებისა, აზიური ფაროსანა ისევ რჩება ბუნებრივი და ხელოვნური ეკოსისტემების სერიოზულ მავნებლად მთელ დედამიწაზე, მათ შორის საქართველოშიც. მწერის პოლიფაგური

ბუნება და ადაპტაციის დიდი უნარი საჭიროებს შემდგომ შესწავლას. ჩვენმა კვლევებმა აჩვენა, რომ მწერის ბიოლოგიის და ეკოლოგიის რიგი საკითხები დასაზუსტებელია საქართველოს რთული გეოგრაფიული და კლიმატური პირობების გათალისწინებით.

შემდგომი კვლევეი შეიძლება განვითარდეს რამდენიმე მიმართულებით, კერძოდ,

მეცნიერული თვალსაზრისით:

- გაგრძელდეს აზიური ფაროსანას *H. halys*-ს ბიოეკოლოგიის დაზუსტება საქართველოს პირობებში;
- გაგრძელდეს მწერის ადგილობრივი ბუნებრივი მტრების გამოვლენა და შესწავლა;
- გაგრძელდეს თხილის, როგორც სტრატეგიული კულტურის, შედარებით გამძლე ჯიშების გამოვლენა რეგიონების მიხედვით;
- მოხდეს აზიური ფაროსანას ადგილობრივი პოპულაციებიდან ახალი ენტომოპათოგენების გამოყოფა;
- განხორციელდეს უკვე გამოყოფილი ენტომოპათოგენური სოკოების კომერციალიზაცია, გამოცდა და გამოყენება ქვეყნის მასშტაბით.
- შეიქმნას სპეციალური ლაბორატორია, სადაც მოხდება ჩოქელას, ასევე სამომავლოდ გამოვლენილი სხვა ბუნებრივი მტრების, ხელოვნურად გამრავლება და მიწოდება ფერმერებისა და დაინტერესებული პირებისადმი.

დისერტაციის შედეგების შემდგომი განვითარება და გამოყენება ხელს შეუწყობს ბიოლოგიური კონტროლის გაძლიერებას აზიური ფაროსანას (და არა მარტო) მიმართ, ქიმიური საშუალებების ინტენსიური მოხმარების შემცირებას, ეკოლოგიურად სუფთა, ჯანსაღი და კონკურენტუნარიანი პროდუქციის მიღებას, გარემოს ეკოლოგიური ბალანსის შენარჩუნებას.

ტექნიკური თვალსაზრისით: ბიოპრეპარატები, რომელიც დამზადებული იქნება ეკოლოგიურად მდგრად ადგილობრივ ბიოაგენტებზე, უფრო ეფექტიანი და ადაპტირებული იქნება გარემოს ცვალებადობის მიმართ.

კომერციული თვალსაზრისით: იზოლირებული და შესწავლილი ენტომოპათოგენური სოკოები შესაძლოა შეთავაზებული იქნას როგორც ადგილობრივ, ასევე რეგიონალურ ბიოპესტიციდების მწარმოებლებისადმი.

კვლევის შედეგები გამოქვეყნებულია მაღალი რეიტინგის საერთაშორისო-სამეცნიერო ჟურნალებში:

1. Kharabadze, N., Chkhaidze, N., Abramishvili, T. et al. (2022). First report of *Hierodula transcaucasica* (Brunner von Wattenwyl, 1878) predation on the *Halyomorpha halys* (Stål, 1855) in Georgia. Int J Trop Insect Sci 42, 3283–3292 https://doi.org/10.1007/s42690-022-00826-2
2. Kharabadze, N., Tsiklauri, N., Burjanadze M., Chkhaidze N (2022): Resistance of Georgian Hazelnut (Corylus L.) to Brown Marmorated Stink Bug – *Halyomorpha halys* (Stål), Journal of Nuts 10.22034/JON.2022.1958636.1171
3. Kharabadze, N. (2022): Review of the Brown Marmorated Stink Bug – *Halyomorpha halys* (Stål) in Georgia: Distribution, Biology and Management, Annals of Agrarian Science 20 (2022) 131-147.
4. Burjanadze, M., Kharabadze, N., Arjevanidze M. (2021): Bover-Ge– mycopesticedes for the control Brown Marmorated Stink Bug - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), Annals of Agrarian Science, Annals of Agrarian Science 19, 199-203 https://journals.org.ge/index.php/aans/issue/view/26
5. Burjanadze, M., Gorgadze, O., De Luca, F., Troccoli, A., Lortkipanidze, M., Kharabadze, N., Arjevanidz,e M., Fanelli, E., Tarasco, E. (2020): Potential of native entomopathogenic nematodes for the control of brown marmorated stink bug *Halyomorpha halys* in Georgia, Biocontrol Science and Technology 30:9,962-974, https://doi:10.1080/09583157.2020.1776217
6. Burjanadze, M., Natalia Kharabadze, N., Chkhidze N. (2020): Testing local isolates of entomopathogenic microorganisms against Brown Marmorated Stink Bug *Halyomorpha halys* in Georgia, BIO Web of Conferences 18, 00006, IV All-Russian Plant Protection Congress, https://doi.org/10.1051/bioconf/20201800006
7. Burjanadze, M., Kharabadze, N., Chkhaidze N. (2020) Entomopathogenic fungi and nematoodes as biocontrol agents of Brown Marmorated Stink Bug – *Halyomorpha halys* in Georgia. Book of abstracts VIII Congress on Plant Protection, p-91. Published online https://doi.org/10.1051/bioconf/20201800006

კვლევის შედეგები აპრობირებული იყო საერთაშორისო ფორუმებზე:

1. Kharabadze N., Chkhaidze, N., Lobjanidze, M., Burjanadze. M. **B**iological agents for the control of Brown Marmorated Stink Bug (BMSB) *Halyomorpha halys* in Georgia. International Conference "Brown Marmorated Stink Bug (BMSB) Phytosanitary Regulatory Framework ". March 11-14, 2019. Tbilisi, Georgia
2. Kharabadze, N., Chkhaidze, N., Burjanadze, M., Lobjanidze, M., Didebulidze, K., Brown Marmorated Stink bug (BMSB) – *Halyomorpha halys* - a new hazard for biodiversity of Georgia. 1st International Scientific Conference. Advances and perspectives of Biodiversity Research and Conservation in Georgia. 20-22 May, 2019, Tbilisi, Georgia
3. Burjanadze, M., Kharabadze, N., Chkhaidze, N., Entomopathogenic fungi and nematoodes as biocontrol agents of brown marmorated stink bug – *Halyomorpha halys* in Georgia. Book of abstracts VIII Congress on Plant Protection, p-91. Zlatibor, Serbia, November 25-29, 2019
4. Burjanadze, M., Kharabadze, N., Arjevanidze M. "Bover-Ge – mycopesticedes for the control Brown Marmorated Stink Bug - *Halyomorpha halys* (stål) (hemiptera: pentatomidae)" 6TH Asia Pacific International Modern Sciences Congress. 2021, Deli, India
5. Burjanadze, M., Kharabadze, N., Arjevanidze, M."Effectivness of Local Isolates of Entomopathogenic Microorganisms to Brown Marmorated Stink Bug *Halyomorpha halys* "3rd International Modern Scientific Research Congress. May 06-08, 2022 / Istanbul Gedik University, Istanbul, Turkiye

Author's declaration

As the author of the submitted doctoral dissertation, I declare that my dissertation is an original work and that materials published, or defended by other authors in it are used in accordance with the proper citation rules.

Natalia Kharabadze

December 2023

Abstract

The following thesis is focused on the study of bioecological characteristics and biological control perspectives of invasive insect pest brown marmorated stink bug (BMSB) - *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae), which become a key pest in Georgia since 2015 year and caused tens of million dollar losses only in hazelnut production.

H. halys feeding was observed on 38 species of plants. Three number of generation were detected in West Georgian conditions. Potential of population density growth was estimated by sex ratio index. Investigation of two local variety of hazelnuts revealed that relative resistance potential of the hazelnut fruit depends on the time of initiation of lignification and amount of the accumulated lignin in shell.

The following local natural enemies have been identified from *H. halys* populations: predators - *Rhynocoris iracundus* (Roda, 1761) (Hemiptera: Reduviidae), *Hierodula transcaucasica* (Brunner von Wattenwyl, 1878) (Mantodea: Mantidae), *Iris polystictica* (Fischer-Walheim, 1846) (Mantodea: Eremiaphilidae), *Dermeste* sp. (Coleoptera: Dermestidae), and entomopathogenic fungi - *Beauveria bassiana*, *Isaria fumosorosea*. As a result of research, adult and nymph *Hierodula transcaucasica* can be used to control *H. halys*. In order to determine the virulence of isolated entomopathogenic fungal strains (MB 101, MB 102, MB 103, MB 104) their enzymatic activities were studied. The strain MB 101 was characterized by the highest enzymatic (chitinase - 0, 6057 U/mL, lipase - 1,564 U/mL and protease - 1,06 U/mL) activity. The strain MB 101 with the highest enzymatic activity was characterized by the highest mortality rate at all the three concentrations $1x10^6$, $1x10^7$, $1x10^8$. For this strain mortality rate against nymphs was 80%, 97%, 99% and against adults - 80%, 93%, and 99% respective to the upper mentioned concentrations. Biological efficiency of local biopesticide Bover-Ge (based on *Beauveria bassiana*) was evaluated and demonstrated 72.0-90.5% mortality against adults and 65% - against overwintering *H. halys*. Additionally, entomopathogenic nematodes *Heterorhabditis bacteriophora* (GEO), *Steinernema borjomiense* (GEO), *Heterorhabditis bacteriophora* (IT) and *Steinernema apuliae* (IT) were able to infect *H. halys* adults and mortality achieved 53.3%, 40%, 95.3%, 93.3% respectively.

It is a global challenge to reduce chemical pesticide application, find natural enemies and develop ecologically safe biological control strategies against *H. halys* that will mitigate biodiversity loss and climate change.

Keywords: *Halyomorpha halys*, bioecology, plant resistance, natural enemies, biological control.

Acknowledgements

We would like to thank our colleagues for their backing and support:

Professor Dr. Dr. h.c. mult. Angelika Ploeger, Sustainable Agriculture and Food Systems (SAFS), Project Director – VW, Kassel University, Germany;

Professor Dr. Dr. h.c. mult. Hartmut Vogtmann - Kassel University, Germany;

Professor Vakhtang Lezhava Rector of the Agricultural University of Georgia;

Professor Dr. Teo Urushadze, Dean of Agricultural and Nature Science School at the Agricultural University of Georgia, SAFS program director from Georgia;

Natia Samushia Vice-Rector of the Agricultural University of Georgia;

Professor Nato Kobakhidze, Ph.D. School coordinator of the Agricultural University of Georgia; **Nino Katcharava** SAFS program coordinator.

I would like to express my gratitude to my supervisors:

Professor Dr. Medea Burjanadze Professor, Senior Research Scientist, Laboratory of Forest Protection, Vasil Gulisashvili Forest Institute, Agricultural University of Georgia

Professor Dr. Michael Rostas, Agricultural Entomology, Department for Crop Sciences, Georg-August-Universität Göttingen

Professor Dr. Nona Chkhaidze Associate prof., Manager of Botany and Plant

Physiology Educational and Research Laboratories, Agricultural University of Georgia

I would like to thank people who helped and gave me important recommendations during my studies:

Professor Dr. Nino Tsiklauri, Senior scientist- S. Durmishidze institute of Biochemistry and Biotechnology, Agricultural University of Georgia;

Professor Dr. Levan Gulua, Head of bachelor program of Biomedicine, University of Georgia;

Professor Dr. Kakha Didebulidze, Professor, Coordinator of Teaching Laboratories, Institute of Veterinary Medicine, Head of Laboratory of Water Microbiology, Agricultural University of Georgia;

Professor Dr. Mzagho Lobjanidze, Professor, Agricultural University of Georgia;

Professor Dr. George Japoshvili, Professor, Director of Institute of Entomology, Agricultural University of Georgia;

Dr. Tea Abramishvili, Head of Laboratory of Phytopathology, Plant Pest Diagnostics Department, State Laboratory of Agriculture (SLA);

I would like to express my sincere thanks to the farmers with whom I performed my field experiments:

Marika Kodua

Vasil Mikaberidze

Finally, would like to thank to my family and my emotional supportive pets.

Table of contents

List of tables

List of figures

Abbreviation

BMSB - Brown Marmorated Stink Bug

CABI - Centre for Agriculture and Bioscience International

CDEs - cuticle-degrading enzymes

CTAB – Cetyltrimethylammonium bromide

DNA – Deoxyribonucleic acid

EPPO - European and Mediterranean Plant Protection Organization

FAO - Food and Agriculture Organization

GEO – Georgia

GlcNAc - N-acetylglucosamine

IJs - Infective Juveniles

IPCC - Intergovernmental Panel on Climate Change

IPM - Integrated Pest Management

IT-Italy

ITS – Internal transcribed spacer

PCR – Polymerase Chain Reaction

PDA - Potato Dextract Agar

SDG - Sustainable Development Goal

SDGs – Sustinable Development Goals

WG – Western Georgia

1 Introduction

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stal.) (Heteroptera: Pentatomidae) is an invasive pentatomid, native to East Asia and spread throughout much of the United States, Canada and Europe, presently a severe agricultural pest. BMSB has continued to spread and establish itself in new Mediterranean and Black Sea regions (Hoebeke and Carter 2003; Lee et al., 2013, Bergmann, 2015).

H. halys is a highly polyphagous pest insect species with a strong dispersal capacity, has a broad host range that includes over 170 plants, many of agricultural importance, including various fruits, vegetables, row crops and ornamentals (Leskey et al., 2012a). Damage worth in millions were done to agricultural crop production in the Mid-Atlantic States of America in 2010. *H. halys* is capable of long-distance flight (Leskey, 2015; Wiman, et al., 2014), walking dispersal (Lee D-H et al., 2014) and frequent movement among crops and wild host plants as they mature during the growing season. It is also a structural pest, with high reproductive output, potentially enabling its spread and success in invaded regions. Serious nuisance pest problems are caused by *H. halys* in the fall due to its aggregatory behaviors in houses for overwintering. The invasive *H. halys* is more successful due to a lack of specific natural enemies, reproduction in large numbers, wide host range, resistance to cold weather, effective overwintering strategies, and increased survival due to global warming (Lee et al., 2012, Leskey et al., 2012b). Since the first report of *H. halys* in Europe more than 10 years ago, the species has recently become an important pest in many agroecosystems (Haye et al., 2015).

Following its first detection in 2014, *H. halys* has become a key pest in many crops in West Georgia. *H. halys* invaded Georgia from Sochi (Russia) (Gapon, 2016). The Black Sea humid subtropical zone of Georgia begins from Gagra and ends in Sarpi. There is abundance of rainfall, (2000 - 2700 mm/y), high humidity (80-100%), warm winter and moderately hot summers. In this area there are widely introduced subtropical crops (citruses, tea, persimmon, Chinese wood-oil tree, etc.) from Indo-China Peninsula and Japan, which are the native regions of *H. halys* (Kharebava, 1967; Beraia, 1984). In area of its natural distribution *H. halys* voltinism variates from 1 to 6 according to climate conditions (Lee, 2013). On the Black Sea

coastline *H. halys* develops two or three generations (Karpun and Protsenko, 2016; Murvanidze, 2018).

Georgia is rich not only in wild plant diversity, but also in agricultural crops. According to National Statistics Office of Georgia, in 2017, 43% of the country's labor force were employed in agriculture sector and about 98% of farm workers are considered self-employed. 43% of Georgian citizens economic well-being is dependent on agriculture and crop production. Nowadays the situation is quite alarming in Western Georgia, where main agricultural crops (maize, vegetables and hazelnuts) are damaged by *H. halys*.

In 2014 hazelnut was Georgia's main agricultural export product, according to data from Georgia's Agricultural Ministry. As one of the country's top 10 export commodities, it is a source of income for more than 50,000 farmers and dozens of processing facilities (Tsintsadze, 2018). Its production and export had decrease dramatically in 2017 - a 54% decrease from the previous year, with a total volume of $54 million (Ministry of Finance of Georgia, 2017).

Nowadays, *H. halys* prevention depends on broad spectrum-insecticides, especially pyrethroids (Bifentrine). This practice disrupts integrated pest management programs, causing outbreaks of secondary pests such as European red mites, wooly apple aphids and San Jose scale, that are normally controlled by natural enemies (Leskey, 2012a). Bifenthrin is a chiral synthetic pyrethroid insecticide that has been commonly used for agricultural and domestic pest control over the past decades. Additionally, bifenthrin can also cause sublethal toxic effects on various non-target organisms, including developmental toxicity, neurobehavioral toxicity, oxidative damage, immune toxicity and endocrine disrupting effects (Yang, 2018).

The chemical treatment measures against *H. halys* pollute environment, kill beneficial insect and have negative effect on environment.

Biological pest control is good alternative for avoiding pesticide use leading to many problems like air, water and soil pollution, health hazards, killing of beneficial organisms and secondary pest outbreak (Sathe, 2000).

There is a number of potential biological control agents against *H. halys* identified in Asia. Among them are egg parasitoids, predators and entomopathogens (Lee, 2013). Usage of entomopathogenic fungi *Beauveria bassiana* is effective measure against *H. halys* (Gouli et al., 2012) and egg parasitoid *Trissolcus japonicus* is one of the effective biological control agents

in its native area (Yang, 2009), but its introduction in West Georgia may be a threat to biodiversity and cause additional problems.

1.1 Research Objectives, Novelity and Scientific Contribution

This dissertation explores bioecological characteristics, natural enemies and biological control of the brown marmorated stink bug - *Halyomorpha halys* in Georgia. Despite the conducted studies, there were no accurate data on the number of *H. halys* generations, the composition of host plants, markers of host plant resistance, local natural enemies and their effectiveness in the conditions of Western Georgia.

This thesis describes the results of laboratory and field study of bioecological characteristics and biological control of *H. halys* in West Georgia. In particular, the objectives of my research were to

1) study some biological peculiarities of *H. halys* (generation, host plant, markers of plant resistance)

2) search and identify natural enemies of *H. halys*

3) estimate biocontrol potential of identified natural enemies and

4) assess efficiency of entomopathogens and entomoparasitic nematodes against *H. halys*.

The results of this study have theoretical and practical outputs and influence the social-economic field, protecting biodiversity and the environment. The acquired knowledge will be used for practical actions to control *H. halys*. The identified local natural enemies of *H. halys* will contribute to the development of local production of biopesticides in Georgia and the Caucasus region as well.

1.2 The outline of the Dissertation

The Structure of Dissertation consists of: 1 - introduction, 2 - literature review, 3 - research methodology, 4 - results and discussion, 5 - conclusion and recommendations chapters. In Chapter One a general introduction is given about distribution, harmful activity, host plants and management of *H. halys*. In Chapter Two extensive literature data around *H. halys* bioecological characteristics, invasion, life cycle in different locations, caused economic losses, chemical and biological control tools are analysed. Methods and design used for the research are described in Chapter Three. Obtained data and discussion are presented in Chapter Four. Conclusions, recommendations and prospects for further development and application of the thesis is given in Chapter Five.

2 Literature Review

2.1 Synonymy, Taxonomy and Nomenclature

Halyomorpha halys (Stal, 1855) (=*Pentatoma halys* Stål (1855), *Poecilometis mistus* Uhler, 1860; *Dalpada brevis* Walker, 1867; *D. remota* Walker, 1867). Distribution area: Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Neu Monggol, Shaanxi, Shanxi, Sichuan, Taiwan, Xizang, Yunnan, Zhejiang. Also recorded from Korea and Japan (Rider et al., 2002).

This species has had a long and confusing history. It has appeared in the literature under *Halyomorpha halys* and all three junior synonyms; in fact, it appears that many workers in Japan still refer to it as *H. mista*. It has also frequently been confused with *H. picus*, an Indian species. Josifov & Kerzhner (1978) determined that only one species of *Halyomorpha* is present in eastern China, Japan, and Korea, and it is different from *H. picus*; the oldest available name is *H. halys* (Stal) (Rider et al., 2002).

2.2 Distribution in the world

In the last 30 years, the species was detected in different parts of the world, mainly in North America and Europe (global distribution recently summarized by Leskey and Nielsen (2018). The first record of established population out of the native range dates in 1996 in Pennsylvania, USA, although prior to its establishment, *H. halys* was reported first time in 1973 (Hoebeke and Carter, 2003). This invasive species has spread across 43 states of the US and two provinces in Canada (Rice et al., 2014).

From 2017 this invasive species has been detected in almost all states of USA, including Alaska and Hawaii, lacking only in Oklahoma and Louisiana (Kriticos et al., 2017; Walgenbach, 2017; EPPO, 2018). In North America *H. halys* is reported also in southern Canada, where it was firstly intercepted in the Province of British Columbia in 1993, when a specimen was found in a shipment originating from Asia (Haye et al., 2015). In South America *H. halys* is recorded from Chile, where it was intercepted for the first time in 2011 and later 2017 near Santiago (Faúndez and Rider, 2017) and finally were found in the Caribbean countries.

In Asia, out of the native range, *H. halys* is quoted for India, reported in 2014–2015 by Nikam and More (2016). However, this record is likely a misidentification with a different species (Cianferoni et al., 2018).

In Oceania some sporadic records are known from New Zealand, when it was observed for the first time in 1999 (Duthie, 2012), Australia, when it was introduced for the first time in 2005 (Walker, 2011), and Guam island, where a single specimen was collected in a hotel room in 2013 (Moore, 2014). In Africa the species is not documented, but the record for Egypt of *H. picus* (Gadalla, 2004) could be a misidentification for *H. halys* according to Aukema et al., (2013) and Hemala and Kment (2017). In the native distribution area, Japan and China, outbreaks are not recorded. Most Complete data on the distribution of this species are given at EPPO Global Database (2022).

2.3 Distribution in Europe

The other continent where *H. halys* has become a pest is Europe, where the oldest records date back to 2004: in that year *H. halys* was found in Liechtenstein (Arnold, 2009) and Switzerland (Haye et al., 2014). Subsequently, it colonized many Swiss territories (Wyniger and Kment, 2010; Haye et al., 2014) and some years later it spread into neighboring countries: in southern Germany in 2011 (Heckmann, 2012) and in France in 2012 (Callot and Brua, 2013, Garrouste et al., 2015, Maurel et al., 2016), also its in Corsica (Kriticos et al., 2017). In 2007 *H. halys* occurred in other European countries, in Italy (Maistrello and Dioli, 2014; Dioli and Maistrello, 2016) and in 2011 in Athens, Greece (Milonas and Partsinevelos, 2014).

Until now, many countries in Europe have been added to the list of those where the *H. halys* occurs: in 2013 it was firstly recorded in Hungary (Vétek et al., 2014) and in Krasnodar, Russia (Gapon, 2016), in 2015 in Austria (Rabitsch and Friebe, 2015), Serbia (Šeat, 2015), Romania (Macavei et al., 2015), in 2016 in Spain (Dioli et al., 2016), Slovakia (Hemala and Kment, 2017), Bulgaria (Simov, 2016), and Georgia (Abkhazeti) (Gapon, 2016), and in 2017 in Slovenia (EPPO, 2018), Turkiye (Çerçi and Koçak, 2017), and Croatia (Šapina Šerić Jelaska, 2018). Moreover, Malumphy (2014) reports that *H. halys* was founded twice in Great Britain, one in 2010 in London, in association with passenger luggage flown in from the USA, and the other in 2013 in North Yorkshire, associated with a consignment of stone imported from China.

Gapon (2016) predicted a high probability of range expansion in the Russian Federation and neighboring countries, at least as far as the entire North Caucasus, the Rostov region, the south of the Volgograd region, as well as neighboring countries: Ukraine, Moldova, Bulgaria, southern Poland, also Armenia, Azerbaijan and Turkiye. These findings are based on the work of Zhu G. et al., (2012), which used the Maximum Entropy methods (MaxEnt) and Genetic Algorithm for Rule Set Production (GARP) methods. The model of *H. halys* distribution shows that more suitable areas for its spread are located between latitudes 30° and 50°, including parts of Europe, North America, Australia, the New Zealand and part of Africa. (Zhu et al., 2012) (Fig. 1).

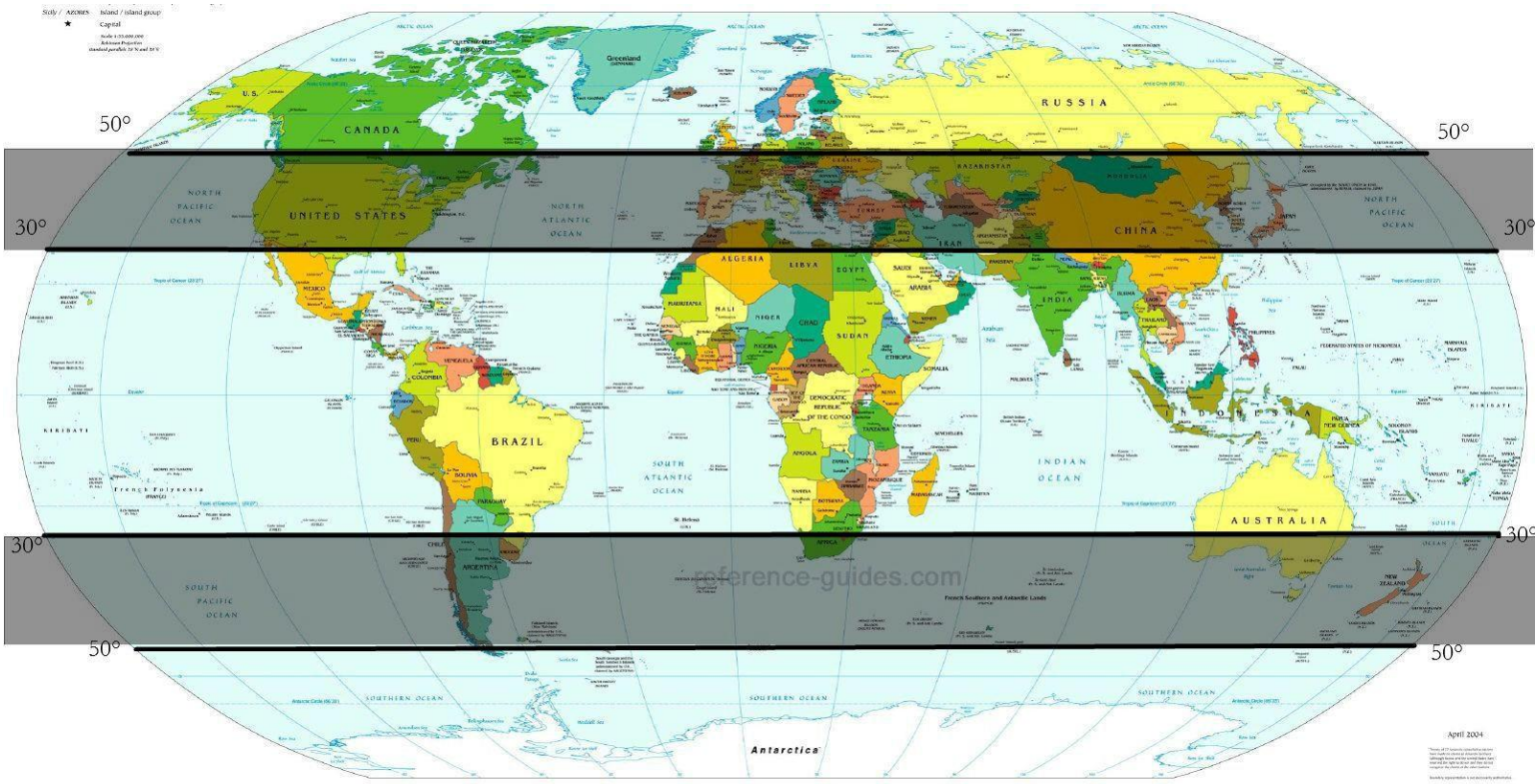


Fig. 1. Suitable areas for *Halyomorpha halys* distribution between latitudes 30° and 50°
Source: Wine for Rookies, Inc

2.4 Distribution in Georgia

In Georgia, *Halyomorpha halys* (Hemiptera: Pentatomidae – formerly EPPO Alert List) was identified for the first time in October 2016. Its outbreak in Georgia was reported in the municipality of Khobi (Samegrelo-Zemo Svaneti region), as well as in Pitsunda (Abkhazia) (Gapon, 2016). However, it is noted that the presence of the bug had already been observed in 2015. This is the first time that *H. halys* is reported from Georgia (Current pest situation evaluated by EPPO on the basis of information dated).

It is not completely clear how the bug entered in Russia. There is an assumption (Gapon, 2016) that this species was brought (probably from Italy) to one of the Russian Black Sea ports with planting material of ornamental plants for landscaping the facilities of the XXII Olympic Winter Games, similar to what happened in the case of the boxwood moth *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Pyraloidea: Crambidae) (Eskin and Bibin, 2014). This assumption is also dictated by the fact that at the same time *H. halys* was not found in the

neighboring countries (Moldova, Ukraine and Turkiye). By this time the bug had spread from Russia to Georgia via Abkhazia region. Based on the available data, it can be assumed that *H. halys* spread throughout the territory of the Krasnodar Territory at a speed of 100–150 km per year (Neimorovets, 2018).

Zhimerikin and Guliy (2014) suggested that the Sochi City Administrative Okrug (territory of Greater Sochi) may be a place appearance of the *H. halys* in Russia. Its larvae were found in the city in August 2014 (Mityushev, 2016). The author of the article studied 1 adult specimen (female) with labeled "Russia, Sochi, Central District, 28.IX.2014. Koval A.G. leg". There are reports that this species appeared in the Sochi region no later than 2013 (Gapon, 2016). From the second half of 2015 mass reproduction of the bug began in the Sochi urban district, which led in 2016 to large losses in the harvest of fruit and subtropical crops (Karpun, Protsenko, 2016).

In 2017-2018 for investigation of hazelnut orchards the *H. halys* were detected in the Guria, Samegrelo, Adjara and Imereti regions of Western Georgia (Kharabadze et al., 2019; Burjanadze et al., 2019, 2020). About distribution bug the same regions, were reported by another scientist from Georgia as well (Meskhi, 2017; Jakely & Nikolashvili, 2019; Dumbadze et al., 2019; Japoshvili et al., 2022). At present insects are invaded and distributed in some municipals of Eastern Georgia, where hazelnut orchards are cultivated (Japoshvili at al., 2022). The distribution of *H. halys* in territories of Georgia is given in Figure 2.

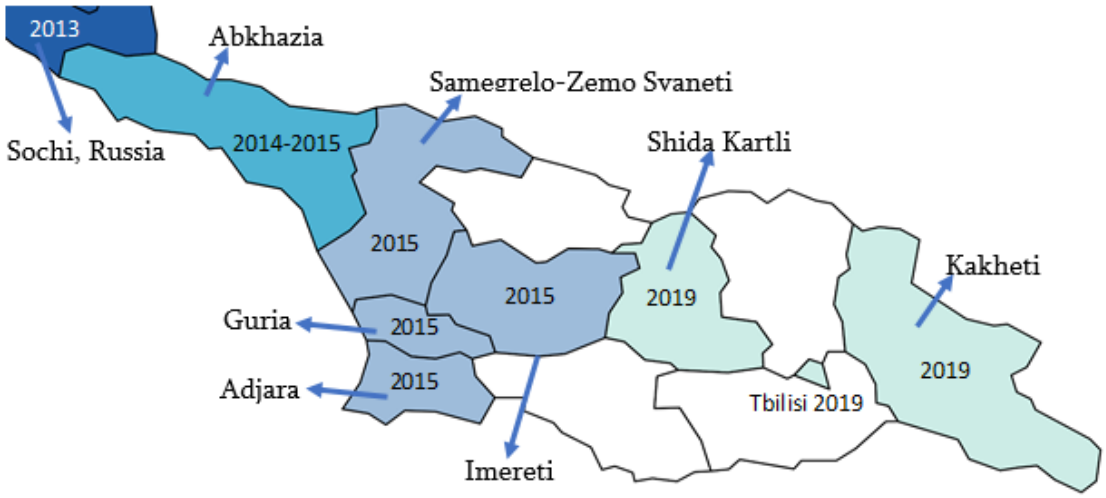


Fig. 2. *Halyomoprha halys* distribution in Georgia according years

The situation of *Halyomorpha halys* in Georgian neighbor countries can be described as follows: In Azerbaijan, it is distributed in the Cheki-Zakatala (Eastern part, bordering Georgia), Lankaran and Absheron zones, where nut (hazelnuts) fruits and subtropical crops are grown (Mamedov, 2018); First record on the *H. halys* in Turkiye was 2017. This agricultural pest was detected in Kemalpaşa District of Artvin Province located in the Eastern Black Sea Region near the Georgian border (Güncan and Gümüş, 2019). However, information about this insect in Armenia no reported, only findings information as a hypothesis its distribution are given on the work of Zhu G. et al., (2012).

2.5 Life cycles

The brown marmorated stink bug, like all stink bugs, is a hemimetabolous insect and a multivoltine species, development from egg to adult takes approximately 40 to 60 days, depending on temperature and photoperiod. After hatching, first instar nymphs may aggregate around the egg clutch before molting and dispersing to feed. Adults can produce multiple egg clutches throughout their lifespan. Winter diapause is a crucial component of the brown marmorated stink bug life cycle. Bugs respond to shortening daylength during fall by entering into diapause. During this period adult reproductive activity ceases as the stink bugs conserve resources to survive the winter. Only adults enter diapause and survive through the winter. Increased temperatures and daylength in the spring signal an end to the dormant period, and adult brown marmorated stink bugs will leave their overwintering sites in search of food. In warmer climates several generations per year are possible, though in most of its North American range the brown marmorated stink bug has one to two generations per year (Penca & Hodges, 2018; 2019).

As a mention *H. haly*s is a multivoltine species (Hoffman, 1931), in some geographical range, the life cycle is commonly characterised by one or two generations (Nielsen and Hamilton, 2008; Lee et al., 2013), but in Southern China four to six generations are assumed to occur (Hoffman, 1931). Pre-reproductive adults overwinter in large number and they often use human houses as overwintering sites, with a documented case of 26,205 individuals in a single house during a 181-day study (Inkley, 2012). Adults entering houses are a strong

nuisance not only for their abundance, but also for the unpleasant odor they emit when disturbed (Haye et al., 2014). This behaviour leads to a variety of pest impacts also for the human life (Kriticos et al., 2017), which add to the problems that *H. halys* causes to agricultural, horticultural and silvicultural hosts (Haye et al., 2015).

In Georgia, two generations of *H. halys* are commonly presented (Jakely & Nikolashvili 2019), as a three-generation reviewed N. Kharabadze (2020) in Western Georgia (WG), that Black Sea region characterized by subtropical climate, with warm weather and humidity, which is favorable for this pest insect and help its development and rapidly growing population.

H. halys biology involves pest development in three stages (egg, nymph and adult phase) and are following: (1). The pest overwinters in the adult (imago) phase; (2). Adults phase copulation begins two weeks after diapause; (3). Eggs are laid by adults and continue at intervals throughout the life of the female to the end. There are observed pyramidal cluster mostly containing 28 eggs (average 26.27, st.dev- 4.20), but we are fined clusters with minimal number of eggs - 12, and maximunm - 32. On average 80.8 % of the laid eggs were hatched successfully. Under optimal conditions one female lays up to 400 eggs. Eggs are laid on the underside of the host plant leaf and they are white color; (4). The first nymphs hatch from eggs after 4-5 days; (5). The pest has 5 nymphal phases. Each last one week, at room temperature according to its change; (6). Adult and nymphs characherizaied with active movement and feeding. They are easy move on the vegetative parts of the plant, fly and feed by Fruits. The pest is characterized by the pierce-sucking mouthparts; (7). An adult insect infests almost all parts of the plant, especially the fruits. The damages part became corroded, hardened and the crop loses its agricultural significance; (8).

There is two (Murvanidze et al., 2018) or three (Kharabadze et al., 2019) generations of *H. halys* in Georgia. The number of generations depends not only on the length of the day, but also on the temperature, because under suitable temperature conditions the development of the *H. halys* is faster (Halye et al., 2014), this process is also facilitated by the diversity of host plants (Acebes et al., 2016) and air relative humidity (Khadka et al., 2020). Therefore, in the humid subtopical regions of WG - Guria and Samegrelo, in 2018 three generations were observed: the first-generation egg-laying began in the early of May and lasted until the middle

of May. Due to worming climate with annual average temperature 12 °C, (Climate Risk Country Profile: Georgia, 2021) and wide range of wild and cultivated host plants, *H. halys* populations develops within 32 days from egg to adult. The second generation emeges in the first ten dayof July and lasted until early August. Third generation occurs second ten dayof August and for mid of October they are entering to diapause.

2.6 Food specialization and harmful activity

H. halys is a broad polyphage and feeds on flowers, stems, leaves and fruits of plants 49 families (Duthie, 2012). In the Sochi region and in Abkhazia 32 plant species from 16 families were recorded, on which the bug fed (Musolin et al., 2017). Loss caused by the *H. halys* in the region of humid subtropics of Russia and West Georgia (Abkhazia), especially noticeable on fruit and vegetable crops: on the surface of fruits and leaves of apple and pear trees in places of punctures necrosis, corking is formed, under the skin – dry cottony tissue, the taste of the fruit deteriorates, the surface becomes bumpy; on citrus and persimmon leads to underdevelopment and premature fall of the fruit; on grapes - the berries do not develop and fall off; on hazelnuts, it damages nuts at the stage of milky-wax ripeness, leading to the cessation of the development of the kernel; on corn grains do not develop. Damage can be aggravated by secondary infections. So, for example, on pepper and tomato fruit rot develops at the puncture sites (Rice, 2014; Karpun and Protsenko, 2016). Bugs also can transmit phytoplasmoses causes pathogen (Jones and Lambdin, 2009).

In a review of the Asian literature (Lee et al., 2013) revealed in native range 106 host plants distributed in 45 families, while Haye et al., (2014) reported 51 hosts in 32 families in Europe and Bernon (2004) quoted 73 species of plants ranging from annual crops to landscape trees only in the state of Pennsylvania. Now, over 275 species of host plants are cited in literature for this bug (Australian Government Department of Agriculture and Water Resources 2017) and it is considered a severe agricultural and horticultural pest (Kriticos et al., 2017). Feeding may occur on leaves, shoots, and stems and even through the bark of trees such as maple and catalpa (Rice et al., 2014). However, both nymphs and adults preferentially feed on developing and ripe fruits and seeds of their host plant (Martinson et al., 2015). This mode

of feeding, and preference for fruits, directly leads to economic damage to a wide range of crops.

In Georgia 2018-2021 years, during observation twelve plants species as host of *H. halys* were identified: *Eriobotrya japonica*, *Laurus nobilis*, *Actinidia sp.*, *Agave sp.*, *Solenostemon sp.*, *Pueraria sp.*, *Rubus canescens*, *Sambucus ebulus*, *Convolvulus sp.*, *Robinia pseudoacacia*, *Tradescantia sp.*, *Pteris cretica*. They were not included as host plants of this insect in CABI, EPPO, and official lists of the Ministry of Environment Protection and Agriculture of Georgia (Kharabadze et al., 2019).

In its native range, it is considered an occasional pest of fruit trees and soybean, Glycine max (L.) Merr. (Fabales: Fabaceae), as well as a nuisance pest during the winter (Hoffman, 1931; Saito et al., 1964; Kobayashi and Kimura, 1969; Chung et al., 1995; Funayama, 1996; Watanabe, 1996; Choiet al., 2000; Tada et al., 2001; Funayama, 2002; Rijal and Sudan, 2018).

Insect damages wild, ornamental and cultivated plants. They mainly feed on pome, stone, nut, legume fruits. The signs of *H. halys* feeding range from the multiple punctures in the hull (endocarp) to the formation of the necrotic tissues on the shell (endocarp), hull (exocarp and pericarp) and the kernel (endosperm) of the fruit (Hedstrom et al., 2014). Damaged fruits have necrotic spots or blotches, grooves and brownish discolorations. In Asia, *H. halys* is considered as causing significant damage to soybean and various horticultural crops. In Japan, apple crops have increasingly been damaged by *H. halys*. Forest trees are also known hosts of *H. halys*. However, in Japan *H. halys* is considered as a pest in nurseries producing seeds of cedar and cypress because it can feed on cones. In the USA, damage caused by *H. halys* was initially reported in suburban or urban environments on woody ornamentals (e.g. *Buddleia davidii, Paulownia tomentosa*) and backyard peach and pear trees. However, in 2006, commercial fruit growers started to report damage in apple and pear orchards in eastern Pennsylvania and western New Jersey. In Pennsylvania, high populations were also found in soybean crops but without significant damage. *H. halys* is considered as a vector of Paulownia witches' broom phytoplasma in Asia.

In addition to plant damage, *H. halys* can be a nuisance to humans because at the end of autumn, adults can aggregate in buildings and houses (on walls, window and door frames) seeking overwintering sites. When disturbed or crushed they discharge a characteristic

pungent odour (unpleasant and long lasting!). In the USA, many homeowners are complaining about this nuisance.

2.7 Damage and Economic Importance

Many studies have shown that stink bugs in the family Pentatomidae can be serious economic pests of food crops and ornamental plants around the world (Hedstrom et al., 2014; Tuncer et al., 2014). Worldwide, this family contains almost 900 genera and around 5000 species (Rider, 2015). Many pentatomids cause significant damage to hazelnut crops in Europe, Turkiye and Georgia (Bosco et al., 2018). In particular, stink bug pests of cultivated hazelnuts (*Corylus avellana* L., Fagales: Corylaceae) occur in the leading commercial hazelnut producing countries of Turkiye (Tuncer et al., 2005) and Italy (Tavella et al., 1997). Studies in Turkiye report feeding damage to hazelnut kernels by *Palomena prasina* L. (Hemiptera: Pentato-midae) in the form of kernel abortion, malformation, and the occurrence of dry, necrotic tissue (Saruhan and Tuncer, 2010). According Tavella et al., (2001), hazelnut in northwestern Italy was damaged by seven species of Pentatomidae.

In Asia *H. halys* causes significant damage to many economically important crops In Japan, outbreak populations of *H. halys* on apple (Yanagi and Hagihara, 1980, Funayama, 2003, Ohira, 2003), pear, plum, satsuma mandarin, and grape (Oda et al., 1980) have been reported, with damage characteristics and levels varying among crops. In 2010, high densities of *H. halys* caused as much as 100% crop loss in some apple and peach orchards in the Eastern USA. (Leskey et al., 2012b, c). In Italy some early maturing pear cultivars damage at harvest reached more than 50% (Bariselli et al., 2016) Economic damage on pepper crops has been reported in Europe from the Canton Aargau in Switzerland (Sauer, 2012). In 2016 52.7–68.6 million US dollars losses in hazelnut crops which caused by *H. halys* in Georgian (National Food Agency, Ministry of Agriculture of Georgia, 2016).

In Georgia, due to lack of specific natural enemies, high reproduction ability, wide host range, and favorable climate conditions, effective overwintering strategies, increased population rate of *H. halys* that caused huge economic losses in agricultural crop production (Burjanadze et al., 2020). In 2017, compared to 2016, revenue from hazelnut exports fell by $

54 million (Ministry of finance of Georgia, https://mof.ge/images/File/biujetis-kanoni2018/II-wardgena/matrica.pdf). In 2019, the fight against *Halyomorpha halys* was declared successful (Ministry of Environmental Protection and Agriculture of Georgia) , which led to an increase in hazelnut production (National Statistics Office of Georgia).

2.8 Natural enemies

Brown marmorated stink bug parasites or predators, entomopathogens have the potential to provide landscape-scale control of this pest in the future. Since the establishment of the, *H. halys* in North America and Europe, there has been a large, multi-group effort to characterize the composition and impact of the indigenous community of arthropod natural enemies attacking this invasive pest.

2.8.1. Native predators

The list of native natural enemies that attack brown marmorated stink bug includes other species of insects, spiders, and even some birds and mammals. However, insects and spiders are largely recognized as the most important group of natural enemies of BMSB (Jones, et.al, 2014).

Wide variety of generalist predators that consume *H. halys* eggs have identified in the USA. The list includes certain species of crickets, katydids, ground beetles, lady beetles, earwigs, ants, assassin bugs, mantids, and jumping spiders, as well as less familiar insects such as minute pirate bugs, lacewings, and damsel bugs.
(https://entomology.ces.ncsu.edu/biological-control-of-brown-marmorated-stinkbug/#predators)

The community of indigenous generalist predators consuming *H. halys* eggs is very diverse, made up of many species with a variety of modes of feeding. Morrison et al., (2016 b) identified four distinct predation "syndromes" on *H. halys* eggs using laboratory observations of over 25 predator taxa: (1) complete chewing—eggs completely removed from substrate; (2)

incomplete chewing—egg shell debris from predated eggs remains on substrate; (3) stylet sucking—presence of a feeding sheath in eggs drained of their contents; and (4) punctured sucking—a hole or slit in hollowed-out eggs. In the laboratory, the most efficient predators were katydids (Orthoptera: Tettigoniidae), ground beetles (Coleoptera: Carabidae), crickets (Orthoptera: Gryllidae), earwigs (Dermaptera: Forficulidae), and jumping spiders (Araneae: Salticidae) (Abram et al., 2017).

Predators attacking *H. halys* adults and nymphs in Europe and North America have not been extensively studied. In one study of the nest-provisioning wasp *Bicyrtes quadrifasciata* (Say) (Hymenoptera: Crabronidae) in the northeastern USA, *H. halys* nymphs made up 96% of the prey provided to developing offspring (Biddinger et al., 2017). Another crabronid wasp, *Astata unicolor* Say, was also observed preying on *H. halys* nymphs. Jones (2013) commonly observed predation of *H. halys* adults and nymphs by the wheel bug *Arilus cristatus* (L.) (Hemiptera: Reduviidae) in Maryland, USA. Predation of adult *H. halys* by praying mantids (Mantodea: Mantidae) has also been observed on a number of occasions in the USA.

Interestingly, only one unpublished study has gathered some information on the consumption of newly emerged (1st instar) *H. halys* nymphs, which are clustered on egg masses for several days and potentially vulnerable as they are relatively immobile and soft-bodied. That study observed the highest predation of freshly emerged first instar nymphs in the laboratory by field-collected Carabidae (89% of nymphs emerged were eaten), predatory Pentatomidae (88%), Salticidae (35%), and Reduviidae (26 %;) unpublished data, Abram et al., 2017).

According to Kharabadze et al., (2022) *Hierodula transcaucasica* Brunner von Wattenwyl, 1878 was observed as a predator of *H. halys* in Georgia. Predation of the adults and nymphs of *H. transcaucasica* was documented after they underwent starvation periods. The 3rd instar nymph of *H. transcaucasica* preyed on the 1st instar *H. halys* nymphs, while adult *H. transcaucasica* attacked adult of *H. halys*. A maximum number of *H. halys* (7 insects) were eaten by an adult *H. transcaucasica* on the first day, and in this case, the same number of nymphs (7 insects) were observed within the first hours of the experiment.

2.8.2. Parasitoids

Since the impact of native natural enemies on invasive *H. halys* populations in Europe and North America is generally low, *H. halys* was identified as a promising target for classical biological control. Surveys for natural enemies that have co-evolved with *H. halys* in its native range revealed that it is mostly attacked by egg parasitoids, among which the samurai wasp, *Trissolcus japonicus*, was identified as the most promising candidate for classical biological control.

In China, *Trissolcus japonicus* (Talamas et al., 2013) a parasitoid wasp species in the family Scelionidae, is a primary predator (Pfeiffer, 2009). Biological control programs against *H. halys* in the United States, Europe, and New Zealand are focused on *Trissolcus japonicus*. in 2014, two adventive populations were found in the United States during surveys to identify which North American parasitoids might be attacking brown marmorated stink bug. (Talamas et al., 2015; Herlihy et al., 2016) Subsequent genetic testing showed these wild populations were self-introduced: they were not related to each other, or to a laboratory strain being studied in quarantine. (Milnes et al., 2016). (Szűcs et al., 2019, Jentsch, 2017, Jentsch, 2019) An adventive European population was discovered during similar surveys in Switzerland in 2017 (Stahl et al., 2018).

Trissolcus japonicus is a tiny wasp that parasitizes the eggs of various stink bug species. It was first collected from China and brought back to quarantine facilities in the US for evaluation, as a potential biological control agent. Host-specific tests have indicated that *T. japonicus* prefers to parasitize *H. halys* eggs over eggs of other stink bug species.

Abram et al. (2017) reported that *H. halys* and other stink bug species data from the USA supports the hypothesis that the relative prevalence of different parasitoid species associated with *H. halys* eggs is habitat-dependent (Okuda and Yeargan, 1988; Herlihy et al., 2016).

Three principal groups of hymenopteran parasitoids attack *H. halys* eggs in invaded areas of North America and Europe: Scelionidae (*Telenomus, Trissolcus*, and Gryon spp.), Eupelmidae (*Anastatus* spp.), and Encyrtidae (*Ooencyrtus* spp.). The host range of the

scelionids were detected, especially Telenomus (podisi group) and Trissolcus spp., tends to be restricted to stink bugs (Hemiptera: Pentatomidae) (Johnson, 1984 b, 1987).

Scelionid parasitoids of stink bug eggs have stereotyped behavior, remaining on the patch for several hours after oviposition and engaging in aggressive inter- and intraspecific contests with other parasitoids (Field, 1998). Eupelmids and encyrtids found attacking *H. halys* are likely to be generalists that attack multiple families of insect hosts and some species are facultative hyperparasitoids (Cusumano et al., 2013; Noyes, 2015).

According to Japoshvili et al., (2022), five species of *Trissolcus* Ashmead (Hymenoptera: Scelionidae) *Trissolcus belenus* (Walker), *T. colemani* (Crawford), *T.* cultratus (Mayr), *T. semistriatus* (Nees) and *T. scutellaris* (Thomson), which are known parasitoids of Pentatomidae, were identified. According this study, four of these *T. belenus* (Walker), *T. colemani* (Crawford), *T. cultratus* (Mayr) and *T. semistriatus* (Nees) are documented for the first time in Georgia, and two *T. belenus* (Walker), *T. colemani* (Crawford) are new records for the Caucasus region. Only *T. cultratus* (Mayr) was obtained from the *H. halys* egg mass. Also, *Anastatus bifasciatus* was recorded from Georgia as a *H. halys* eggs parasitoid (Kereselidze, et al., 2018) and as a native species considered for potential biocontrol in Europe (Haye et al., 2015).

2.9 Entomopathogens

Entomopathogenic microorganisms are one of the promising naturel enemies for control many invertebrate pests. In this case, less information documented about pathogens of *H. halys.*

First description a unique microsporidian species that infects the green stink bug, *Chinavia hilaris* and the brown marmorated stink bug - *H. halys* first were observied in US. This microsporidium belongs in the genus Nosema, which historically has been characterized by diplokaryotic life stages. This microsporidium is apparently Holarctic in distribution. Nosema maddoxi is native to North America and has also been found in China and South Korea; in North America it infects native stink bugs and *H. halys*.

Morphological, ultrastructural, and ecological features of the microsporidium, together with a molecular phylogeny, establish a new species named *Nosema maddoxi* sp. nov. (Hajek, 2018).

A microsporidian pathogen discovered in Georgian of *H. halys* has been identified as *Nosema maddoxi*. Investigations were carried out during different seasons in three regions of West Georgia in 2018–2019. (Kereselidze, 2019) The highest prevalence of *N. maddoxi* was detected among overwintered adults collected in May in the Guria region. Molecular study was confirmed that this specie is Nosema *madoxi* (Kereselidze et al., 2020).

First report on entomopathogenic fungi species that infects *H. halys* were observed in Guria and Samegrelo region of Western Georgia. In different *H. halys* populations, adults developed mycosis symptoms on the body were observed. Three isolates of the entomopathogenic fungus *Beauveria bassiana* and one of *Isaria fumosorosea* were identified (Burjanadze et al., 2020). Isolated species were studed in morphological and carried out in molecular identification (Burjanadze et al., 2018).

2.10 Management and control

Current management tactics for *H. halys* mostly rely on insecticide applications (Rice et al., 2014; Kuhar and Kamminga, 2017). As indigenous parasitoids and predators adapt to *H. halys* and exotic parasitoids continue to spread and establish, biological control is expected to become an increasingly important component of integrated pest management programs.

At present, there are no published studies of how landscape factors or proposed alternative on-farm management practices (e.g., attractand-kill—Morrison et al., 2016 a; trap crops—Mathews et al., 2017; border and alternate-row spraying—Leskey et al., 2012; Blaauw et al., 2015; insecticide-incorporated nets—Kuhar et al., 2017) influence indigenous natural enemies and their impact *H. halys* population growth. To assess the effect of these pest management practices on biological control services, future studies will need to integrate accurate measurements of population-level impact with chemical, behavioral, and invasive species ecology.

Numerous methods have been used to demonstrate the relative effectiveness of a broad range of insecticides against *H. halys* adults and nymphs, including treated glass surfaces

(Nielsen et al., 2008b, Lee et al., 2012, Leskey et al., 2012c), direct-contact topical applications (G. K., unpublished data), bean dip feeding bioassays (Kuhar et al., 2012e), and field efficacy trials (Kuhar et al., 2012a,b,c,d; Leskey et al., 2014). Active ingredients that have been most effective include several pyrethroids (bifenthrin, permethrin, fenpropathrin, and beta-cyfluthrin), neonicotinoids (dinotefuran, clothianidin, and thiamethoxam,), carbamates (methomyl and oxamyl), the organophosphate acephate, and the organochlorine endosulfan. Unfortunately, these insecticides are generally broad-spectrum in their activity; they can be hard on natural enemy populations and quite disruptive to integrated pest management programs (Leskey et al., 2012b).

Hazelnuts orchards growers in the Black Sea region, Colchis Lowland of WG, chemical insecticide applications were used. USAID helped Georgia in combat against the problems caused by stink bug and donated $3.5 (Meskhi, 2017). State control program against *H.* halys were obtained since November 2016. In Georgia within this program about 110 000 ha of land were treated by pyrethroid insecticides (Bifetrin) per year. Infested plots are treated by thermal fog - with low-volume spraying technology. In 2017, 58,416,8 ha of land were treated, in 2018, 790 000 ha and in 2021 a total area of 37,236 hectares has been treated (https://agenda.ge/en/news/2021/2359).

Also, 21 000 pheromone traps (Tracky), were placed for the monitoring purposes, 100 000 phereomones placed for the “attract and kill” stations in the external perimeters of the forests and villages through the country. Large scale farmers were supplied with pyrethroid (Bifetrin) insecticides. Information campaign is carried out systematically, brochures are printed, video clips are made. A hotline has been opened which can be used for getting comprehensive information and consultation around *H. halys.* In pursuit of the BMSB monitoring, 8000 pheromone traps were placed throughout the country and 28,818 'attract and destroy' stations and 5,754 pheromone clamps where been installed" (https://agenda.ge/en/news/2021/2359). Also, about Bioecological features *H. halys* and it control mechanisms in Adjara Region of WG, use pheromone traps for the monitoring were report in the publication of Dumbadze and all (2019).

According to Burjanadze et al., (2021) against *H. halys* local production of mycopesticiede, trade mark- Bover-Ge™, were tested in laboratory and field condition. It was

registered by National food agency (NFA) of Georgia as a biopesticide, which is based on local strain of entomopathogenic fungus *Beauveria bassiana*, isolated from soil of high mountain of Caucasus region. Bover–Ge were tested at a concentration of 1×10^8 conidia/ml against adults, where in laboratory 93.3%, in field 87% and in migration stage 64% mortality were observed.

The potential of native entomopathogenic nematodes (EPN): *Heterorhabditis bacteriophora* (HRB, GEO) and *Steinernema borjomiense* were compared to Italian strains *H. bacteriophora* (HRB, IT) and *S. apuliae* and evaluated agains adults of *H. halys* in laboratory. Effectiveness of *H. bacteriophora* (GEO, IT) ranged 53-95.3%, from S. *borjomiense* and *S. apuliae* 40-60% were observed. The present study provides further insights in selection of promising EPN strains to be used against *H. halys* (Burjanadze et.al,2020).

2.11 Sustainable Development Goals

This PhD research contributes to the achievement of the above listed United Nations Sustainable Development Goals (SDGs). Using ecological friendly tools is a promising approach. Natural enemies, bio-pesticides, special techniques such as banding trees, using aggregation pheromones can reduce amount of pesticide application. The BMSB can't be eliminated totally from our ecosystems, but biological control tools can make their population under damaging level. Biological pest control is good alternative for avoiding pesticidal use which lead many problems like air and water pollution, health hazards, killing of beneficial organisms, secondary pest outbreak and environment pollution (Tukaram Vithalrao Sathe, 2000). The development of ecological friendly management strategies against BMSB are urgently needed for preserve biodiversity (SDGs 12, 14, 15), water (SDGs 6,14), animals, human and environment (SDG 30), eliminate hunger and economic issues caused by BMSB (SDGs 2, 8), mitigate climate changing (SDGs 12, 13,17).

3 Research materials and methods

3.1 Experimental design

Bio-ecological traits of *H. halys*

- Phenology of *H. halys*
- Sex ratio and eggs hatching rate

H. halys and host plants relationship

- Host plants
- Pathological changes in damaged plants by *H. halys*
- Relative resistance markers against *H. halys* damage

Natural enemies and biological control of *H. halys*

- Sdudy of local natural enemies
- Identfication and estimation local predators against *H.halys*
- Isolation and identification of entomopathogenic fungi
- Characterisation of isolated entomopatogenic fungi (enzymatic activity)
- Estimate biological effectivity of the local and alien entomopatogenic microorganisms (fungi, nematodes) against *H. halys*

3.2 Biomass collection

Plants and insect samples were obtained in 2018-2021, from different geographical areas of three regions: Guria, village Ninoshvili (42°02'24.8"N 41°57'21.9"E, 73 m. a.s.l., Samegrelo,village Darcheli (42°25'14.4"N 41°39'17.6"E, 10 m a.s.l.) and Imereti, Vani (42°05'39.9"N 42°30'12.5"E, 60m a.s.l.)representing different agro- and forest (adjesent) ecosystems of Georgia. Allocated experimental plots were 15 ha, 1 ha, 0.5 ha accordingly.

Alive individuals of *H. halys* were attracted by using, "attract-and-kill" stations baited with the insect's aggregation pheromone and collected by hand or with an insect net and placed in cotton sacks. Pheromone traps were placed at the perimeter and center of the plot to estimate the size of the *H. halys* population. Collected plant and insect samples was placed in transport cooler bag and transferred to the laboratory.

3.3 Morphological study of *H. halys*

To estimate visual key characteristics (body and rostrum sizes, shape, color) study of Wyniger and Kment (2010) were used. The length of the stylet was measured in second and third instar nymphs, and in adults of *H. halys*, according to Rahman & Lim (2017). Ten individuals of each life stage were assessed.

3.4 Egg hatching rate

Naturally occurring stink bug egg masses detected in the field were brought into the laboratory. Observed under the microscope and number of hatched and unhatched eggs counted. In case the egg cluster was not hatched, it was placed in petri dish with wet cotton swab and observed for 5 days. An egg that contained an embryo and had a dark unhealthy color was considered unhatched. Egg hatching rate was calculated for each cluster as the ratio of the total number of eggs in a cluster to the number of unhatched and/or hatched eggs. During investigation period 29 egg clusters was found in total.

3.5 Sex ratio

Adult insects from overwintered populations from Samegrelo and Guria municipalities were observed under microscope. To determine their sex ratio the Bremer formula was used (Drakhovskaia M. 1962):

$i = f / m + f$

f - Number of female insects in population

m -Number of male insects in population

3.6 Phenology of *H. halys*

To determine generation number of the *H. halys* following studies were done: 1.the climatic data of the humid subtropical zone of Georgia were calculated and compared to the climatic requirements of *H. halys*; 2. Recorded the dates of eggs obtained under natural conditions; 3. The data were compared to the literature (Karpun, Protsenko, 2016).

Emergence of the *H. halys* from overwintering were observed in Guria municipality. Adults were collected in October (n = 400). Collected insects were kept in a cardboard box (40x50x40cm) filled with folded papers. Box had exit slit where insects could move. The box containing the adults was placed in room on the wooden shelf. The number of overwintering insects was calculated based on the dead individuals that remained in the box by the end of April.

For the establish number of generations of *H. halys* in west Georgia, following design were used. The branch with one egg mass were wrapped by the mash bag. Eggs hatching and nymph development due to the adult stage were monitored every 24 hours on a daily.

Adult of I generation were monitored until oviposition. On the next step of experiment, the net was opened, removed other insects and eggs, left only one mass of eggs, and monitored to develop II generation which lays eggs (III generation). Observation on the III generation were continued until overwintering *H. halys.*

3.7 Study of host plants of *H. halys* in Georgia and their interaction

Surveys were performed in polyculture areas of Western Georgia, municipalities of Guria, Samegrelo and Imereti in 2018-2021 years (Guria, villadge Ninoshvili, 42°001'58" N, 41°056'28" E. Samegrelo, villadge Darcheli 42°25'04.9"N 41°39'02.1"E, and Imereti, Vani 42°09440'31" N, 42°50306'79" E). Each area included different crops and natural habitats, both uncultivated corridors and forest margins. Plants were randomly selected regardless of species and surveyed by using visual inspection (leaves, limbs, and tree trunks). Plants were inspected additionaly, in case *H. halys* were found on them, to estimate densities of each life stage observed (egg, nymph, and adult).

For the host plant recognize during vegetation period (early/mid-April, mid-/late June, and late September) wild and cultivated host plants were inspected for the presence of *H. halys*. Stink bugs were sampled by visual inspection followed by scouting branches or stems with a cane on a beating sheet (0.7 × 0.7 m). Due to the different size and shape, the plants were visually inspected for 1 min and then scouted on sheet five times (sampling unit).

For the study insect-plant interactions, laboratory and field experiments were used. In the field experiments hazelnut trees, hazelnut fruits were examined.

The two most commonly grown native hazelnut cultivars Berdznula (*C. avellana*) and Tita (*C. colchica*) were used in the experiments. Twenty-five individual trees of each variety were selected, and 50 clusters of nuts were taken from the south-exposed side of the plants. The number of nuts in each cluster was 2.2 ± 0.2 for Berdznula and 2.4 ± 0.4 for Tita. Immediately after sampling, nut damage categories were determined. Additionally, we measured the number of healthy hazelnut kernels, shell thickness and lignin content from visually healthy hazelnuts (II category) (Fig.1). Nut clusters were collected in 2019-2020 (from June till August) in the growth stages "kernel fully expanded" and "kernel mature" (phenology according to Hedstrom et al., 2014; Khomasuridze, 1978; Sichinava, 2005; Mirotadze, 2011). Information on precipitation and air temperature conditions was obtained from the Agrometeorological Bulletin of the Georgian National Environment Agency (nea.gov.ge 2021). Agrometeorological

data were measured at Poti meteorological station (42°09′0″N 41°40′0″E), located 16 km from the experimental field site.

3.8 Damage categories of hazelnut, shell thickness and lignin content

External signs of hazelnut damage caused by *H. halys* were assessed and divided into two damage categories. Category I: numerous perforations visible on surface of nutshell, the nut is strongly withered, and the nutshell has longitudinal splits, fungal infection is occasionally observed. Category II: no perforations of the nutshell surface visible, the nut appears healthy from the outside. Category II nuts were further assessed by cracking the shell open and determining the health status of the kernel inside.

Hazelnut shells of both cultivars were assessed for thickness and lignin content. Nuts were collected in the developmental stages "kernel fully expanded" and "kernel mature" (Hedstrom et al., 2014).

The thickness of each shell (20 nut for each variety) was measured (Khomasuridze, 1978) at its widest dimension around the nut's center. The thickness of the unripe nutshell was measured by using a light microscope (МБР-1) with a micrometer (МОВ-1-15Х ОМО USSR).

Lignin in hazelnut shells (25 nuts for each variety) was assessed microscopically (in growth stage "kernel expression begins") and gravimetrically (in phases "kernel fully expressed", Mature) measuring. For assessing the lignin formation in shells from immature hazelnuts, thin slices (15 μm) of the shell were prepared with a microtome in stage of "kernel express begins" (Microtome Leitz Germany 661) and then dyed. The slices were stained with pale pink safranin solution (1 g l-1 w/v) for 24 h. The staining intensity corresponded to the degree of lignification; tissues lacking lignin remained unstained. Following the staining treatment, shell slices were washed with 50% (v/v) ethanol, acidified with a few drops of 1% (v/v) acetic acid (Japaridze, 1958) and studied under a light microscope (МБР-1). Acid-insoluble lignin content was quantified using the Klason method (Ayeni et al., 2015, Lin and Dence, 1992). For the complete hydrolysis hazelnut shells were ground with a coffee grinder (VitekVT 1540) to a particle size of 1-4 mm. Samples of hazelnut shell powder (1 g) were

treated with 10 ml of 72% sulfuric acid for 24 h. The samples were diluted with 140 ml portions of water and autoclaved at 125 °C, 1.5 atmosphere for 40 min. Mixtures were filtered with a glass fiber filter (SS GF 52 × 47 mm) and washed with distilled water. The filters with the acid-insoluble lignin (Klason lignin) were dried at 103°C, cooled in the desiccator and weighed.

3.9 Natural enemies

3.9.1 Predators

Mantids and *H. halys* were collected in the humid subtropical regions of western Georgia (Guria) and the dry subtropical regions of eastern Georgia (suburbs of Tbilisi) in 2018-2020.

The collected insects were identified by morpological characteristics (Romanowski et al., 2019; Battiston & Massa, 2008). Mounting and morphometric measurements were performed according to the Manual of praying mantis morphology, nomenclature, and practices (Brannoch et al., 2017).

3.9.2 Entomopathogenic fungi

3.9.2.1 Isolation

Collected adults of *H. halys* with visual symptoms of fungal diseases were transferred to the laboratory. Infected insects, at first were examined for under stereomicroscope (Olympus SZ61 Stereo Microscope 0.67x - 4.5x). For the identification of entomopathogenic fungi, symptomatic insects and pathological material were incubated for five days in plastic vials for the moisture atmosphere. Fungi isolated from the body of insects were cultivated on Potato Dextrose Agar (PDA) and incubated at 23 ± 2 °C for 12-15 days. For the following morphological stability isolates were submitted to several rounds of purification.

Isolates of fungi were cultivated on Potato Dextract Agar (PDA) and *Beauveria* selective media (BSM) for 10-14 days at 23 ± 2 °C, until they developed features permitting their identification to species or genus level (Humber, 1997; Inglis, 2012).

The conidia were stained with lactophenol-cottonblue and examined with light and phase- contrast microcopy, to accurately detect morphological characteristics (Zuzi, S120; magnification 400x, 1300x) for entomopathogenic fungi (Rehner et al., 2011; Evlakhova, 1974).

3.9.2.2 Morphological identification

Colony descriptions and measurements were determined from cultures grown on full strength potato dextrose agar (PDA) at 23 ± 2 °C in darkness at 14 day from inoculation. colony coloration, hyphae and conidia were described (Evlakhova, 1974; Roberts, 1992; Humber, 1997). Microscopic measurements of conidiogenous cells and conidia were taken from PDA cultures at 5–15 days and images were acquired with a light microscope. Terminology for conidial shape follows (Vellinga, 1988).

3.9.2.3 Molecular identification

For DNA extraction fungal isolate were inoculated in liquid medium 100 mL potato dextrose broth (PDB, 24 g/L), and incubated at 24 ± 1 °C in the dark for 5 to 7 days for biomass growth. Mycelia was harvested with vacuum, immediately placed in -20 for 4 h, and then placed in lyophylizer for 18h.

Mycelia was powdered in a Retsch miller (MIXER MILL MM 200). The DNA was extracted from 40-50 mg of powder with the CTAB extraction method described previously (Brandfass and Karlovsky, 2006).

The nucleic acids were purified using isopropanol, pellet was washed with 70% ethanol, dried and the DNA was then precipitated with isopropanol, dried and suspended DNA in 50 µl of TE buffer (10 mM Tris.HCl, 1 mM EDTA, pH 8.0).

The purity of DNA was evaluated by horizontal electrophoresis on 1.5% (w / v) agarose gel, stained with containing ROTHI stain (J. Sambrook, D.W. Russell 2001). The ITS regions of the ribosomal DNA were amplified by PCR with specific primers for with ITS 1 (5′-TAGAGGAAGTAAAAGTCGTAA-3′) (Toju et al., 2012) ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990) primers.

The PCR reactions were carried out in a final volume of 25 µl, containing DNase-free water, Buffer 1X, 2 mM magnesium chloride, a set of 150 µM dNTPs, 0,2 µM each of the primers, Taq polymerase 5U/ µl and 1 µg of the extracted DNA. Thermocycler (peqSTAR 96X Universal Gradient) was used for carrying out of polymerase chain reaction. Typical cycle conditions were as follows for the ITS4/ITS5 - initial denaturation is 95 °C -3 minutes, followed by 35 cycles (95 °C - 30 sec, 55 °C -30 sec, 72 °C -1 min.) final 72 °C for 10 minutes. The obtained amplicons were analyzed by gel electrophoresis on 1,5% Agarose with 1,5µl ROTHI stain/25 ml agarose.

The sequencing of the amplicons (PCR products) was performed by the sequencing service of Macrogen Europe, Germany. The sequences obtained were analyzed and the consensus sequences were generated using the Bioedit (Hall, 1999) and compared with the sequences hosted in the NCBI (http://www.ncbi.nlm.nih.gov/BLAST) and Fungal Barcoding (http://www.fungalbarcoding.org) databases.

Phylogenetic trees were constructed with sequences from the ITS region selected from the NCBI-GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) corresponding to the main species within the genera Beauveria and Isaria.

For the analysis were considered reference sequences, data from published papers or sequences from fungal isolates deposited in reference collections. A sequence of the ITS region from *Lecanicillium lecanii* (accession number Genbank CBS101247) was used to root the trees.

3.9.2.4 Enzymatic characteristics

The extracellular cuticle-degrading enzymes (CDEs) such as chitinase, protease, and lipase activities of fungal isolates were evaluated as described by Nahar et al., (2013) with some modifications. These fungal isolates at a concentration of 10^7 spores/ml were inoculated into

250ml Erlenmeyer flasks containing 100ml of chitin medium, and the flasks were incubated on a rotary shaker at 150 rpm for 12 days at 25 ± 1 °C. The medium composition was: 3 g/L KH_2PO_4; $K2HPO_4$, 1.0; $MgSO_4$, 0.7; $(NH4)_2SO_4$, 1.4; NaCl, 0.5; $CaCl_2$, 0.5; yeast extract, 0.5; bacto-peptone, 0.5; chitin, 5.0; and olive oil, 5ml/l. The enzyme extractions and assays were carried out after 24h of incubation up to 12 days. Flasks were removed from the incubator on alternative days, and the broth was centrifuged at 8000 rpm for 25min at 4 ± 1 °C to extract clear supernatant. The supernatant was considered as crude enzyme extract and used for enzyme assays.

Lipase activity was determined by using olive oil and gum arabicas substrate according to the methodology of Pignede et al. (2000) The substrate emulsion was prepared with olive oil (50ml) and gum arabic (50ml, 10% (w/v), Himedia) in the ratio of 1:1. The reaction mixture contained 1 ml of crude enzyme extract, 5ml of substrate emulsion and 2ml of 50mM phosphate buffer, pH 6.8. The reaction mixture was incubated for 1h at 37 °C with constant shaking, and the reaction was terminated with 4 ml of acetone–ethanol (1:1) containing 0.09% phenolphthalein as an indicator. Enzyme activity was determined by titration of the fatty acids released with 50mM sodium hydroxide. One unit of lipase is the amount of enzyme that released of 1mol of fatty acids per min. All the enzyme assays were carried out in triplicates at alternative days.

Protease activity was measured using casein (10 g casein in 100ml of 0.2mM sodium carbonate buffer, pH 9.7) as a substrate (Vyas and Deshpande, 1989). The reaction mixture contained 1 ml of casein, 1ml of sodium carbonate buffer, pH 9.7, and 1 ml of crude enzyme extract. Moreover, the reaction mixture was incubated at 35 °C for 20min. The reaction was terminated by adding 3ml trichloroacetic acid (2.6ml 5% trichloroactic acid) + 0.4ml 3.3 N HCl). The absorbance of the TCA soluble fraction was measured at 280nm. One unit of enzyme corresponds to 1mol of tyrosine per min.

Chitinase activity was estimated in the culture supernatant by using acid-swollen chitin as the substrate. (Vyas and Deshpande,1989) To prepare acid-swollen chitin, 10 g of chitin powder was suspended in 300ml of chilled o-phosphoric acid (88%, w/v) at 4 ◦C for 1h with occasional stirring. The mixture was then poured into ice cold distilled water and filtered through Whatman filter paper. Acid swollen chitin was repeatedly washed with 1% (w/v)

sodium bicarbonate solution, and the pH was adjusted to 7. The solution was homogenized in a Waring blender (1min), and the concentration of acid swollen chitin was adjusted to 7mg/ml by adding 50mM acetate buffer, pH 5.0. The reaction mixture for chitinase assay contained 1 ml of 0.7% swollen chitin, 1ml of 50mM acetate buffer, pH 5.0, and 1ml of crude enzyme extract that was incubated at 50 ◦C for 1h. The N-acetylglucosamine residues (GlcNAc) produced was estimated at 520nm according to the methodology of Somogyi.One unit of enzyme activity was expressed as 1mol of GLcNAc per min.

3.10 Biological control of *H. halys* by predator

3.10.1 Predation rate of adult mantis *Hierodula transcaucasica*

To establish the predation rate of mantis *Hierodula transcaucasica*, an adult female was placed together with ten individuals of *H. halys* in experimental cages (30 x 36 x 30 cm). The results were recorded daily and added the same amount of *H. halys* to the cage as was eaten by the mantis, then the number of *H. halys* in the cage was maintained unchanged daily - up to ten. The plant *Phaseolus vulgaris* (Fabaceae) in a pot was placed in the cage, then sprayed by water, so on its leaves produced water droplets, which was used by mantis.

3.10.2 Hatching nymphs of mantis and its predation in the laboratory conditions

Eggs of *H. transcaucasica* were obtained under laboratory conditions. A branch of the tree was placed in the cage, where *H. transcaucasica* made an ootheca. First hatched nymphs from the ootheca were observed at 23±2 ºC, ~45% RH in May 16. Aphids collected mainly on plants growing in nature (Rosa, Malus, Pyrus, Berberis) were used for nymph's feeding. The nymphs

were grown until 3rd instar. Air temperature and humidity were recorded using a digital psychrometer (TFA Dostmann / Wertheim).

Three nymphs of the mantis (3rd instar) after being grown in the laboratory were transferred in field. Newly hatched *H. halys* nymphs and eggs were found on hazelnut (Corylus sp.) and *Robinia pseudoacacia*. After 42 hours of starvation, experimental nymph of mantis we placed with twentyeight newly hatched nymphs of *H. halys* in the same environment in the cage for the per mantis nymphs. Plastic containers (15x10x8 cm) were used as a cage. The results were recorded within 3 hour-intervals for 24 hours.

3.11 Biological control of *H. halys* by entomopathogenic nematodes in laboratory

3.11.1 Selection and rearing of entomopathogenic nematodes

In experimental trials, four entomopathogenic nematode strains were used: infective juveniles (IJs) of the two Georgian strains *Heterorhabditis bacteriophora* (HRB, GEO) (Gorgadze & Lortkipanidze, 2006) and *Steinernema borjomiense* GS-BOR (Gorgadze et al., 2018) and IJs of two Italian strains, namely *H. bacteriophora* (HRB, IT) IH-LU1 and *Steinernema apuliae* ISMS3 (Tarasco et al., 2015).

Nematodes were reared in last instar *Galleria mellonella* (L.) (Lepidoptera, Pyralidae) at 25°C according to procedures described in Kaya and Stock (1997). The best diet developed by Birah et al., (2008) for *G. mellonella*, comprising wheat flour (130 g), wheat bran (130 g), milk powder (130 g), maize flour (97.5 g), yeast powder (97.5 g), wax (26 g), honey (195 ml) and glycerol (195 ml) was used. Following harvest, nematodes were stored at 13°C for less than 2 weeks before bioassay.

3.11.1.1 Infectivity bioassay on *H. halys*

In laboratory assays (22°C and 80% RH), the four EPNs strains were used in the following three concentrations: 1000, 500 and 200 IJs/ml. The experiments were conducted, following standard procedures (Glazer & Lewis, 2000; Stock & Goodrich-Blair, 2012), in 9-cm Petri dishes lined with a filter paper moistened with a volume of 5 ml/filter of water containing the nematode inoculum. Control Petri dish received 5 ml of water only. Five adults of *H. halys* were placed per Petri dish, with 3 replications per EPN treatment. Numbers of alive and dead *H. halys* were counted 3, 4, 5, 6, 7, 8, and 10 days post-application.

3.12 Biological control of *H. halys* by entomopathogenic fungi

Suspension preparation. Fungal suspensions of the isolates were prepared from 4-week-old cultures grown on PDA at 23±2°C, with good sporulation, using distilled water containing 0.01% (w/v) Tween 80. The obtained suspension was filtered through two layers of sterile muslin into a sterile 50-mL plastic tube, to remove the medium and fungal debris and was then shaken for 5 min using a vortexer for homogenization. The concentration of spores in the suspensions from each fungus was determined using a hemocytometer and adjusted to two concentrations of $1x10^7$ and $1x10^8$conidia ml^{-1} for bioassay (Humber R (1997). The suspensions were used for *H. halys* bioassays in laboratory and field experiments.

Bioassey. The Adults and nymphs of target insects - *H.halys* performed for the bioassay and was treated with fresh culture suspension of: *Beauveria bassiana* - MB 082, MB101; MB102; MB104; Isaria sp. - MB 011, *Isaria fumosorosea* - MB 103 , Metarhizium sp. - MB 077, from collection of EPF, Agricultural University of Georgia and fungal cultures - ARSEF 8318 (*Beauveria bassiana*), ARSEF 8319 – (Metarhizium sp.) from Agricultural Research Service Collection of Entomopathogenic Fungi were used for comparison. For the bioassay, insects were sprayed suspension for inoculation and treatment. Treated insects brought in petri dishes which than was attached on the tree and covered by parafilm that insects can't move outside.

On the top of petri dishes were catted and covered by the net, to get closer to environmental conditions for the experimental trees we are used persimmon, hazelnut.

For establish effectiveness of EPF as a compared variance chemical pesticide "Bi 58 new" (Dimethoate) and a control water treatment were used.

3.12.1 Mycoinsecticide Bover-Ge

The bioformulation is based on a local Georgian strain of *B. bassiana*-024 from high mountain soil of Caucasus Range, supported by molecular identification in CABI-UK, gave a unique cultural number - IMI # 501797 and keep in CABI Genetic Recourse Collection (Burjanadze et al., 2019).

Bover-Ge (powder): was registered by National food agency of Georgia as a biopesticide in 2019 (Burjanadze et al., 2021).

The product was tested at two concentrations ($1x10^7$ and $1x10^8$ conidia/ml) against stink bug adults under laboratory conditions.

3.13 Data Analysis

For statistical analysis of the obtained results, we used the following tests: T-test to determine the difference between the shell thicknesses in different varieties. An F-test was performed to determine the variation in shell thickness of the Berdznula and Titavarieties. Chi-square test was performed to determine the relationships between hazelnut varieties and damage categories, as well as between shell thickness and damage categories.

To determine the morphological nomenclature of praying mantis (Mantodea) and establish the principal difference between the morphometric data of the mantis individual's ANOVA was used. The feeding intensity of the mantis was checked statically by days and hours using Kaplan-Meier survival method. Also, mean and median confidence intervals for survival time were calculated. The calculations were performed at IBM SPSS Statistics 23.

All fungicidal mortality data were corrected for control mortality Abbott's formula (1925) in Laboratory. In field conditions mortality were assessed according Drakhovskaia (1962). The percentage of larvae mortality for each concentration was analyzed using one-way ANOVA; means were separated by Turkey's mean separation test. Mortality was considered statistically significant ($P<0.01$). The Kaplan–Meier survival analysis technique was used to determine both the mean survival and the median lethal time (LT50), the number of days until 50% of insects were dead, for each treatment (GraphPad prism 9.1).

The mean, minimum, median, maximum, standart deviation and standart error of chitinase, protease and lipase activity were analysed by using the SPSS 23.0 software. In the experiments all strain was cultivated in three replications.

Recorded nematocidal mortality data were corrected for mortality in the control group using the Abbott equation (Abbot, 1925). Data were analysed by IBM SPSS 23.0, using a probit analysis method to determine the lethal concentration (LC50) for the different treatments. The mortality data were transformed to probits, while the concentrations were transformed into Probit log10 (dose). Before analysis LC50 values were estimated from the probit lines. For the determination of lethal time (LT50) probit analyses, the method of Finney (1971) was used. Calculation of the lethal concentration (LCs) at their 95% confidence limits (CLs) was based on an accurate estimation of log (LC) variances (Hayes & Kruger, 2014). The Kaplan–Meier survival analysis technique was used to determine both the mean survival and the median lethal time (LT50), the number of days until 50% of insects were dead, for each treatment (SPSS 23.0). In order to calculate significant differences between doses and exposure times, an Onaway analysis of variance (ANOVA) using the SPSS 23.0 software package at $P < 0.01$ and $P < 0.05$ levels was carried out.

4 Results and discussion

4.1 Biomass collection

Route and recognize researches conducted in Samegrelo (village Darcheli), Guria(village Ninoshvili) and Imereti (Vani) regions of West Georgia (Table 1). Field experiments were carried out in Guria and Samegrelo regions (Fig. 3, 4).

Table 1. Investigated *H. halys* spread regions of West Georgia 2018-2021

Region	Geographical location (lat. N, long. E)	Altitude (meter)	Habitat		Year
			Habitat	Sub-habitate	
Samegrelo	42°25'14.4"N 41°39'17.6"E	10	Cultivated	Hazelnuts	2018 2019 2021
Guria	42°02'24.8"N 41°57'21.9"E	73-200	Cultivated Wild	Hazelnuts	2018 2019 2021
Imereti	42°05'39.9“N 42°30'12.5"E	60	Cultivated	Hazelnut	2018 2019 2021

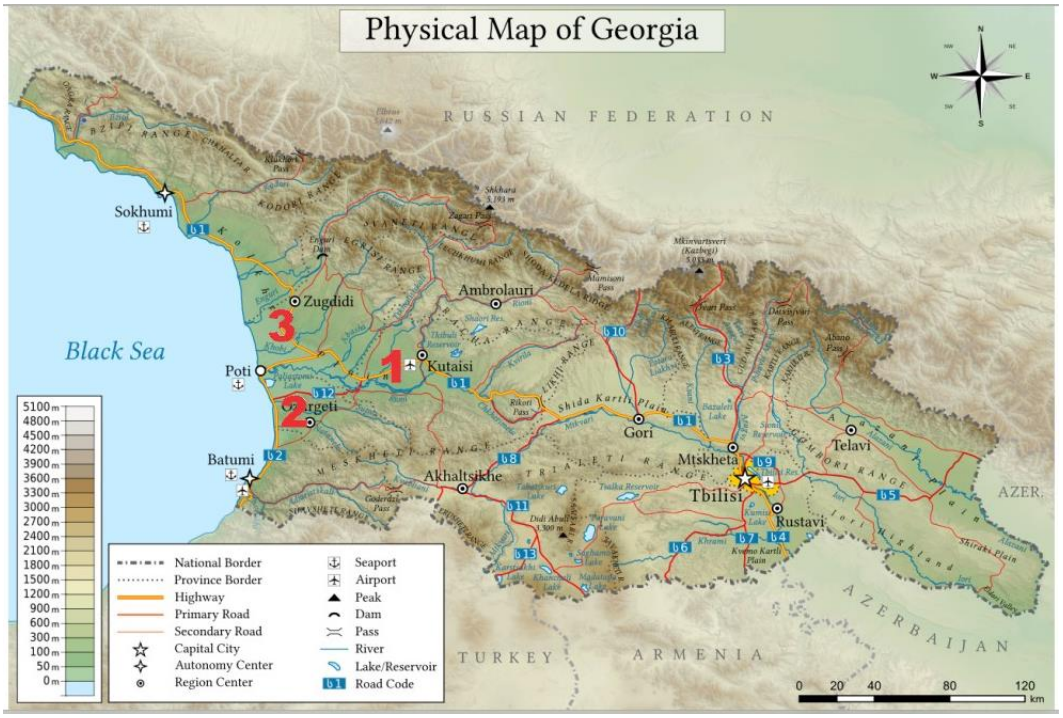


Fig. 3. Investigated locations of West Georgia 1-Imereti, 2-Guria, 3- Samegrelo, 2018-2021 years

Fig. 4. Experimental plots in 1-Imereti, Vani (42°05'39.9"N 42°30'12.5"E), 2 – Guria, Ninoshvili (42°02'24.8"N 41°57'21.9"E) and 3-Samegrelo, Darcheli (42°25'14.4"N 41°39'17.6"E), regions

4.2 Phenology of *H. halys*

Based of comparision climatic data of the humid subtropical zone of Georgia to the climatic requirements of *H. halys* development and recorded the dates of eggs masses obtained under natural conditions it is possible to develop three generations of *H. halys*. (Table 2).

Table 2. Expected phenology of *H. halys* in humid subtropical climate zone of Georgia

Generation	Months an decades																										
	I	II	III	IV			V			VI			VII			VIII			IX			X			XI	XII
				1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
I	◊	◊	◊	◊	◊	▪	▪	●	O	O	O	Δ	Δ													
II												●	●	O	O	O	Δ	Δ								
III																	●	●	O	O	O	Δ	◊	◊	◊	◊

◊–diapause; ▪ overwintered insect; ● - eggs; O- nymph; Δ – imago

4.3 Sex ratio of *H. halys*

Sex ratio in *H. halys* overwintering populations 2018-2021 was determined. Every year female insects were greater than male but highest number of females observed in 2021. In 2021 was expected increase of population density (Fig 5).

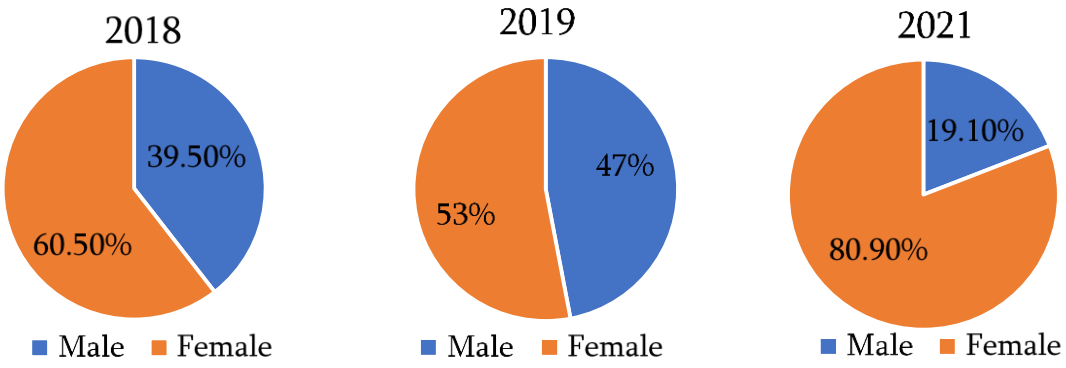


Fig. 5. Sex ratio of overwintered *H. halys*

4.4 Egg hatching

In the studied locations, *H. halys* eggs were found on nuts (Corylaceae), acacia (Fabaceae) and kiwifruit (Actinidia). More than 60% of egg clusters were found on hazelnut (Fig.6) (Table 3).

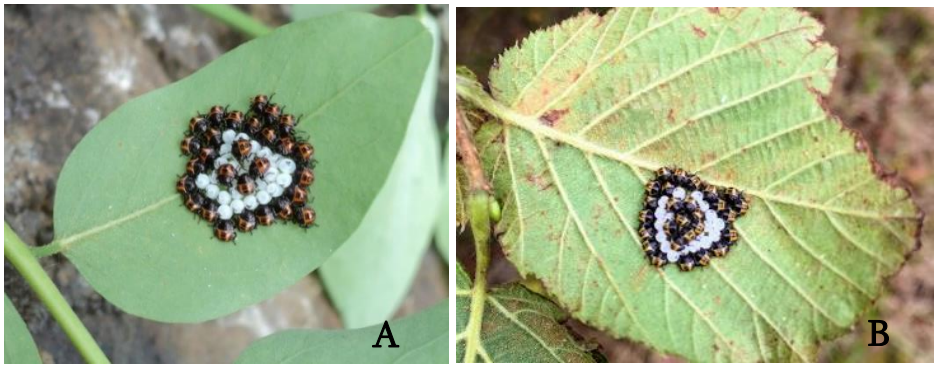


Fig. 6. Hatched egg masses found on A - *Robinia pseudoacacia* and B - Corylus spp.

Table 3. Egg masses on the host plants and eggs hatching rate 2019

Plant	Mean number of eggs in cluster+st.dev	Successfully hatched %
Hazelnut	26.1+4.6	78.05
Acacia	28+0	87.5
Kiwifruit	24+0	100

In 2019 75% of eggs were successfully hatched, in 2020 hatching rate reached 79% (Fig. 7).

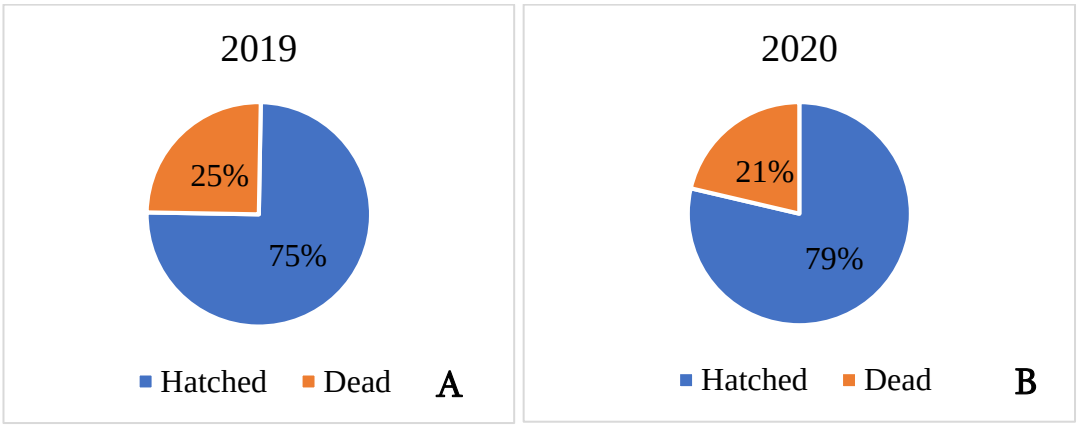


Fig.7. Proportion of successfully hatched and unhatched eggs according to years

4.5 Host Plants

H.halys feeding was observed on 38 species of plants representing, 31 genera and 23 families. Also 5 varieties of *Corylus pontca,* 2 varieties of beans and 2 varieties pears (in generally 9 variety). In Georgia observed some of the host plants: *Eriobotrya japonica*, *Laurus nobilis*, *Actinidia sp., Agave sp., Solenostemon sp., Pueraria sp., Rubus canescens, Sambucus ebulus, Convolvulus sp., Robinia pseudoacacia, Tradescantia sp., Pteris cretica*, which are not included EPPO and CABI *H. halys* host plant lists (2020).

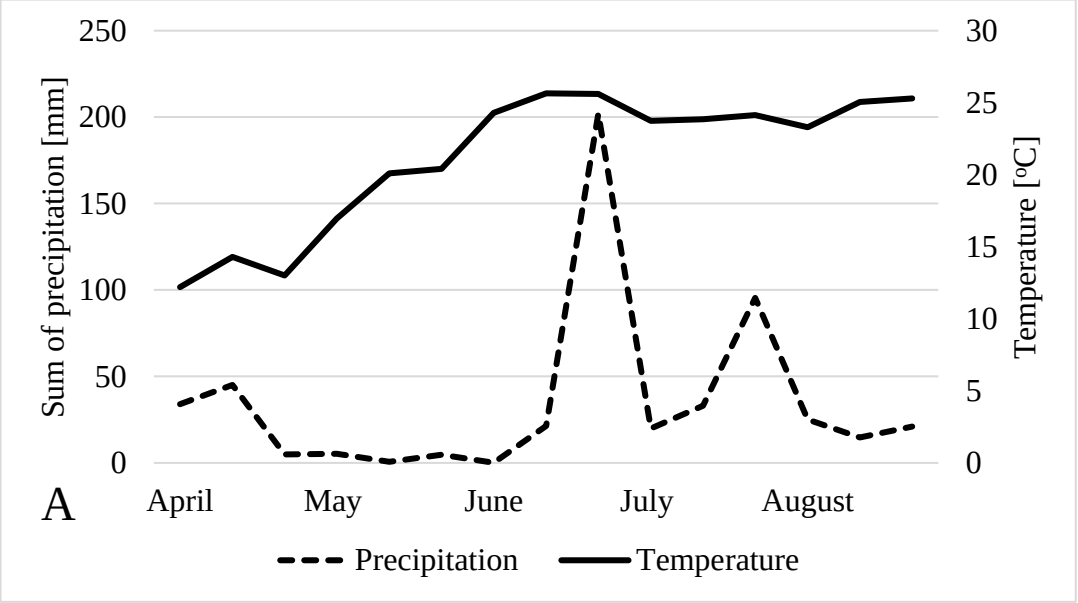
Table 4. Host plants of *H. halys* according Family, Genus, Species and Varieties.

Family	Genus	Species	Varietas
Adoxaceae	*Sambucus*	*Sambucus ebulus* L.	
Asparagaceae	*Agava*	*Agava americana*	
Asteraceae	*Helianthus*	*Helianthus annuus L.*	
Actinidiaceae	*Actinidia*	*Actinidia sp.*	Gigantea
Betulaceae	*Corylus*	*Corylus colchica*	Berdznula, Dedoplis Titi, Chicha, Tsiteli, Surebis, Nemsa
		Corylus avellana *Corylus imeretina*	
Convolvulaceae	*Convolvulus*	*Convolvulus arvensis* L.	
Commelinaceae	*Tradescantia*	*Tradescantia virginiana* L.	
Euphorbiaceae	*Aleurites*	*Aleurites cordata* (Thunb.) Steund	
Ebenaceae	*Diospiros*	*Diospiros kaki* (Thunb.)	Khachia
Elaeagnaceae	*Elaeagnus*	*Elaeagnus umbellata* (Thunb.)	
Fabaceae	*Phaseolus*	*Phaseolus vulgaris* (L.)	all varieties
	Pueraria	*Pueraria hirsuta*	
	Robinia	*Robinia pseudoacacia*	

Fagaceae	*Castanea*	*Castanea sativa* L.	
Lamiaceae	*Lamium*	*Lamium album* L.	
Lauraceae	*Laurus*	*Laurus nobilis* L.	
Moraceae	*Morus*	*Morus tatarica*	
	Ficus	*Ficus carica*	Tetri
Poaceae	*Zea*	*Zea mays*	
Pteridiaceae	*Pteris*	*Pteris cretica L.*	
Rosaceae	*Eriobiotrya*	*Eriobiotrya japonica* (Thunb.) lindl.	
	Malus L.		Zamtris
	Prunus	*Prunus cerasifera*	
		Prunus persica	
		Prunus domestica	Alibukhari
	Pyrus	*Pyrus communis* L.	Nikato, Saivanobo
		Pyrus communis L., subsp. balanseae	
	Rosa L.	*Rosa sp.*	ornamental
	Rubus	*Rubus fructicosus* L.	
Rutaceae	*Citrus*	*Citrus* nobilis Lour.	Unshiu
		Citrus limon (L.) Osbeck.	Meyer
		Citrus sinensis (L.) Osbeck.	Washington
Solanaceae	*Solanum*	*Solanum lycopersicum* L.	
Urticaceae	*Urtica*	*Urtica dioica* L.	
Vitaceae	*Vitis*	*Vitis labruska* L.	Isabella (Adesa)

4.6 Meteorological conditions and phenology of hazelnut

The vegetation periods from April till September in 2019 and 2020 differed in average air temperature and precipitation. In particular, 2019 was characterized by lower rainfall and higher temperatures compared to 2020 (Fig.8). Starting by the end of April, adult *H. halys* emerged from overwintering sites, and after maturation feeding, produced the first generation at the end of May-beginning of June). The second ten day of April 2020 was 0.5°C warmer, and the third ten daywas 1.5 °C colder and rainy compared to the corresponding periods in 2019. In spring 2019, the first insects were observed in the 2nd ten dayof May, and in 2020 in the 3rd ten dayof May. In spring 2020, the number of overwintering insects was greatly reduced - we caught five individuals. At the end of April, the nut is in the embryonic phase, after it goes through the following phenological phases: c. the presence of an embryo, d. kernel expression begins, pericarp formation, e. kernel fully expressed, end of sclerification, f. kernel matures, and harvests. Variety Berdznula undergoes phenophases 7-10 days earlier than Dedoflis Titi.



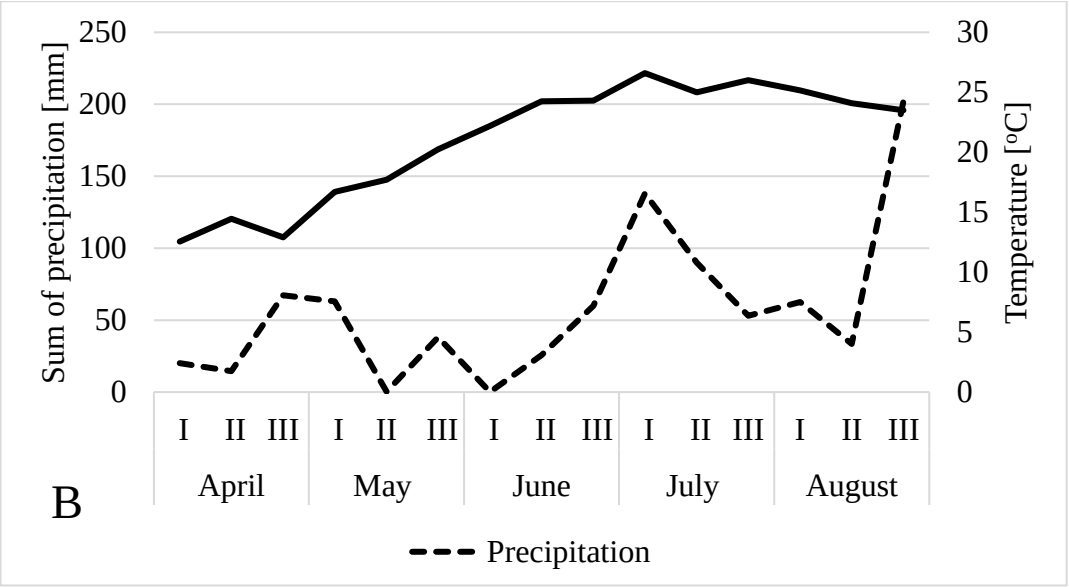


Fig. 8. Monthly average air temperature and precipitation A-2019, B-2020

4.7 Hazelnut fruit damages

Hazelnut fruit feeding damages were observed in both cultivars Tita and Bedznula, with the number of injuries in 2019 exceeding those of 2020. The visually healthy fruit of the Berdznula was 60%, while in the Titawas only 43% (Fig.9). Some of the visually healthy fruits were also found to be damaged by the insect.

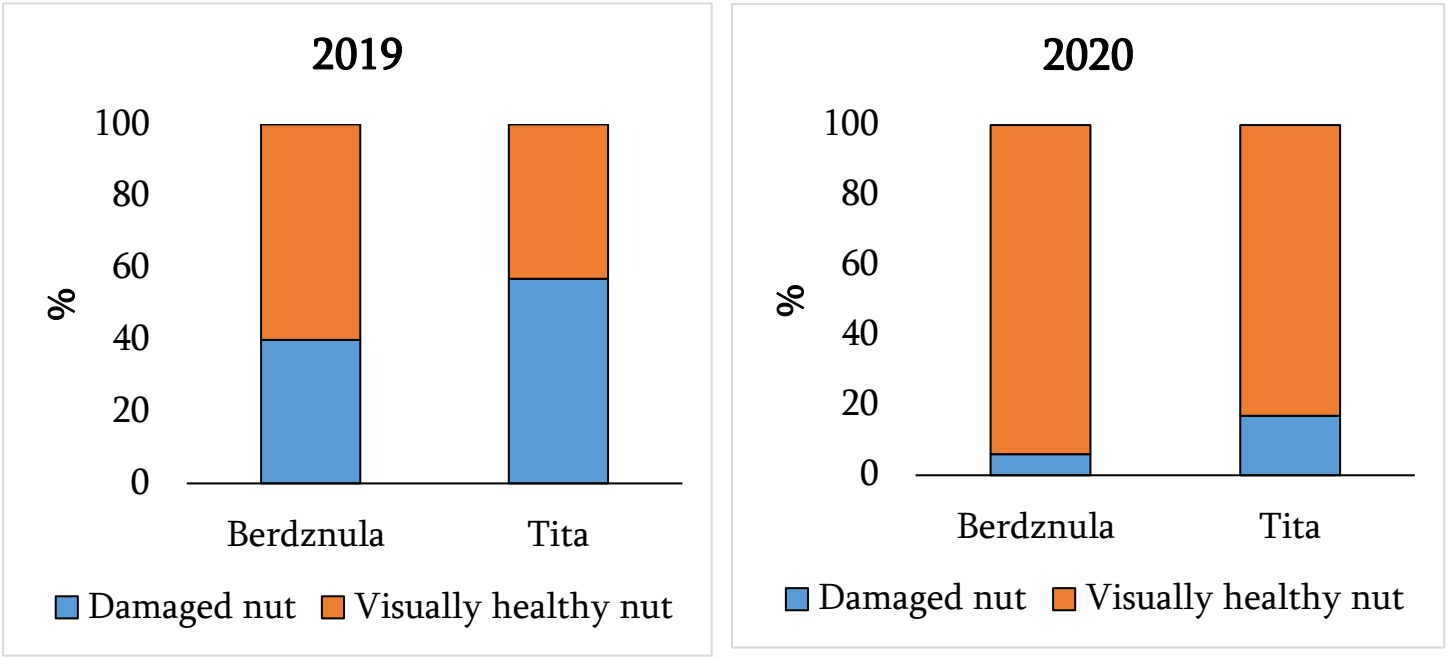


Fig. 9. Proportion of visually healthy and damaged nuts in Berdznula and Tita variates according 2019-2020 years

The symptoms of the damages were the same as in general: more damage was reported in both varieties in 2019, although the percent of kernel damage in the Berdznula was less than one year in both species (Fig. 10). In the kernel of a healthy, ripe nut (Fig. 10. A) the embryo (1) and its nutrition tissue in the cotyledon (2) are clearly visible. As a result of *H. halys* feeding, both the embryo and the reserve tissue (Fig. 11. B.C.) were necrotic.

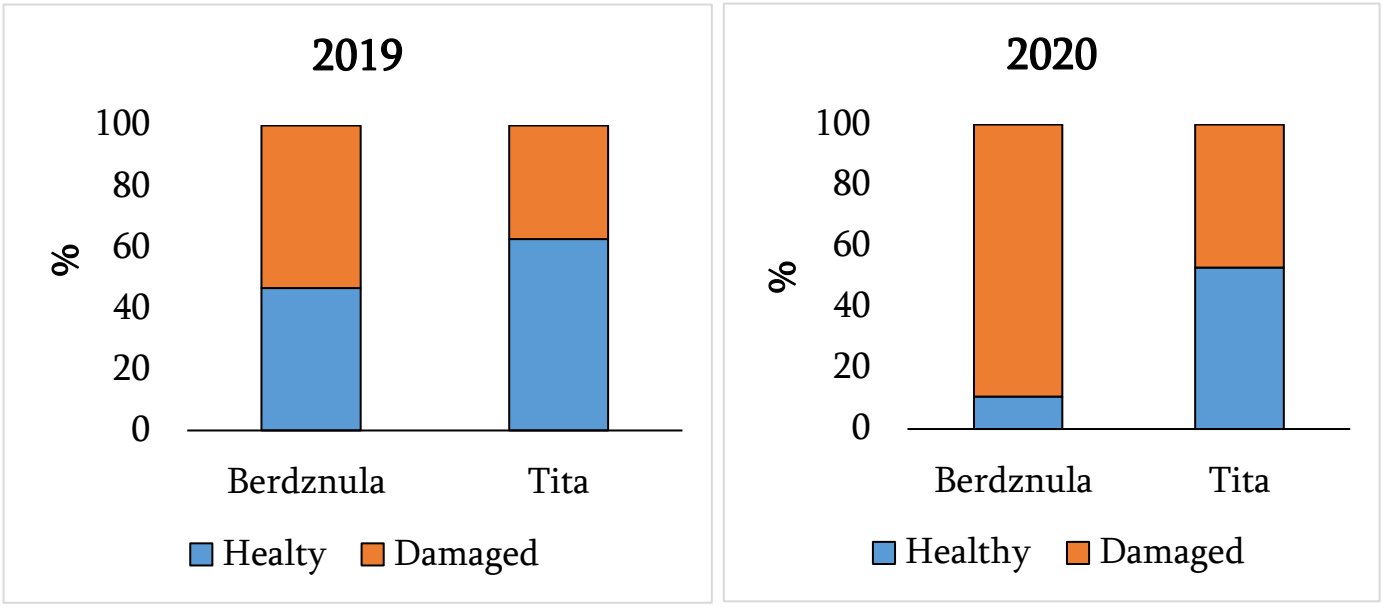


Fig. 10. Proportion of damaged kernels in Berdznula and Titavarieties healthy looking nuts after splitting open 2019-2020 years

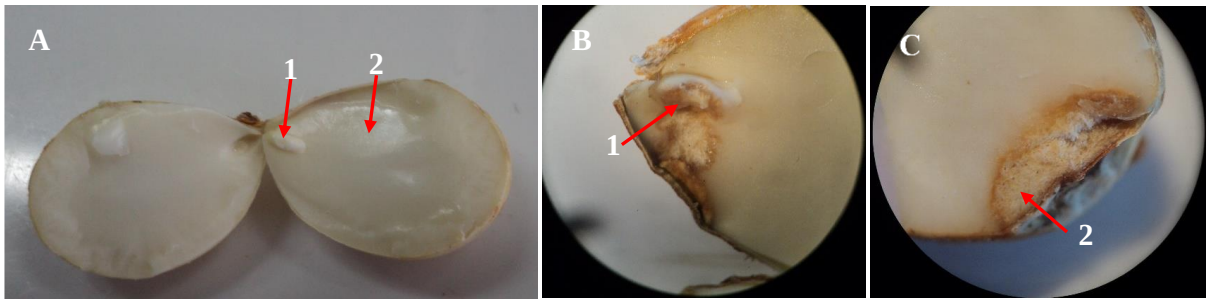


Fig. 11. Healthy (A; natural size, 1. embryo 2. nutrition tissue in the cotyledon) and corking damage symptoms (B-embryo, C- lowest part of the kernel; x20) of hazelnut

After splitting open visually healthy nuts a proportion of the kernels were found to be damaged by the insect (Fig.11). In 2019, on average 60% of all nuts were healthy looking, of which 32.0 ± 1.16% showed healthy kernels in cultivar Berdznula. In 2020, 94±1.16% visually healthy kernels were observed, of which healthy kernels were 84±2.08%. In the case of Titain 2019 visually healthy fruits reached 43.3±0.88%, of which only 16.3±0.88 had healthy kernels. In 2020 83±0.58% were visually healthy, of which 39±1.73% had a healthy kernel (Fig.10). Chi-square test shows that there is a dependency between hazelnut variates (Berdznula and Dedoflis Titi) and damage categories (p-value = 0.048464 < 0.05).

4.8 Nutshell thickness

The thickness of the shell depended on the hazelnut variety and its developmental stage. In the first ten days of June when the first and second instar nymphs of the first *H. halys* generation were observed, most nutshell growth was completed and shells had reached their maximum thickness. Thicker nutshells were observed in the cultivar Berdznula than in Tita (Fig. 12).

Bedznula nuts in the stage" kernel expression begins "(16.06.19) had a shell thickness of 1.21 ± 0.04 mm (mean ± SE) while the shell thickness of Titanuts was 0.75 ± 0.02 mm (mean ± SE). At the second measurement 70 days later, hazelnuts were in the growth stage "kernel mature "(27.07.19). At this time point, the thickness of Berdznula shells had reached 1.23 ± 0.11 mm (mean ± SE), while the thickness of the Tita shells was 0.76 ± 0.03 mm (mean ± SE).

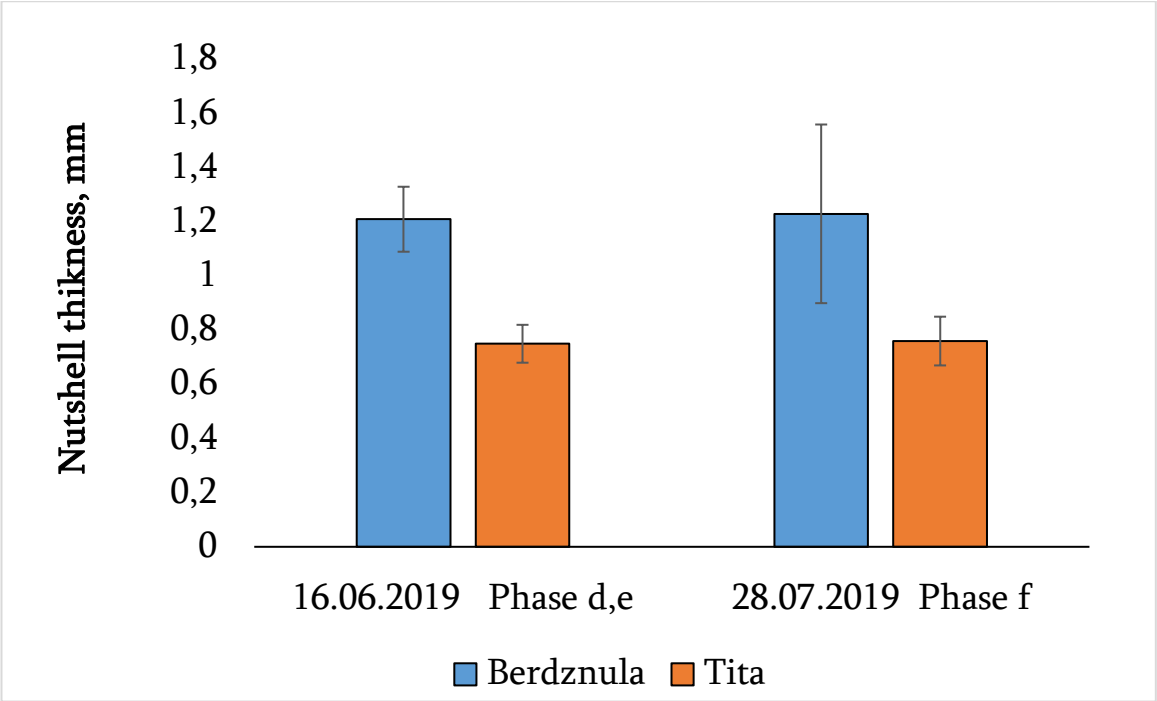


Fig. 12. Changes in the thickness of the hazelnut nutshells during ontogenesis

A comparison of nutshell thickness between varieties Tita and Berdznula assumes that variances for the two cultivars are significantly different (*p-value* = 0.008). The t-statistic is 4.281 with 18 degrees of freedom. Also, corresponding two-tailed *p-value* is less than 0.05.

4.9 Lignin formation in a nutshell

In the first days of June, the pericarp cells in the nutshell started to lignify. Enhanced thickening of the cell walls, the breakdown of cell content, and the formation of dead sclerotized tissue were found. Microscopic observation showed that cultivar Berdznula had a thicker lignified zone than Tita (Fig. 3, arrow 3). In the pericarp of Berdznula lignification was father advanced and clearly expressed as dark Bordeaux-red coloring (Fig. 13 A), while in Tita lignification was still in an early stage (light pink coloration, Fig. 13 B).

A quantititive analysis of the lignin concentration showed that in 2019, hazelnut shells in Berdznula had accumulated significantly more lignin than shells in Tita (Mann-Whitney U-test, $U = 50.5$, $p < 0.001$, $n = 19$).

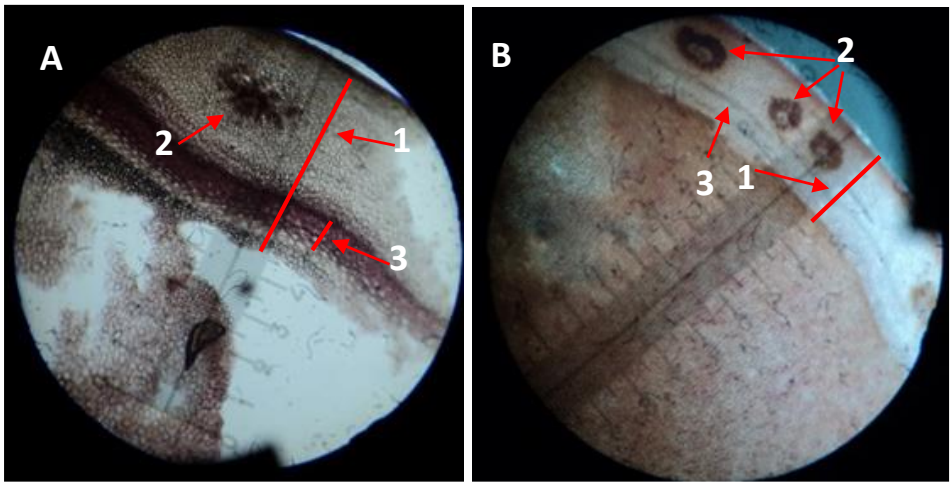


Fig. 13. Micrographs of safranin stained hazelnut shells (A. Berdznula, B. Tita). 1. Pericarp, 2. Conductive vein in pericarp, 3. Lignified zone. x 24. (10.06.19)

However, no difference in nutshell lignin concentration was found in 2020 (Mann-Whitney U-test, U = 116.0, p = 0.063, n = 18-20). Differences between lignin quantity Berdznula and Tita 8% were observed in 2019.

The lignin quantity of the cultivar Berdznula in 2019 reached 51.79±0.001% (mean± Std. error) and in 2020 was less than the previous year 39.21±0.001%. 2019 Titaconsisted of 43.95±0.003% of lignin and 40.13±0.0003% in 2020 accumulated (Fig.14). No significant difference was observed between both cultivars in 2020.

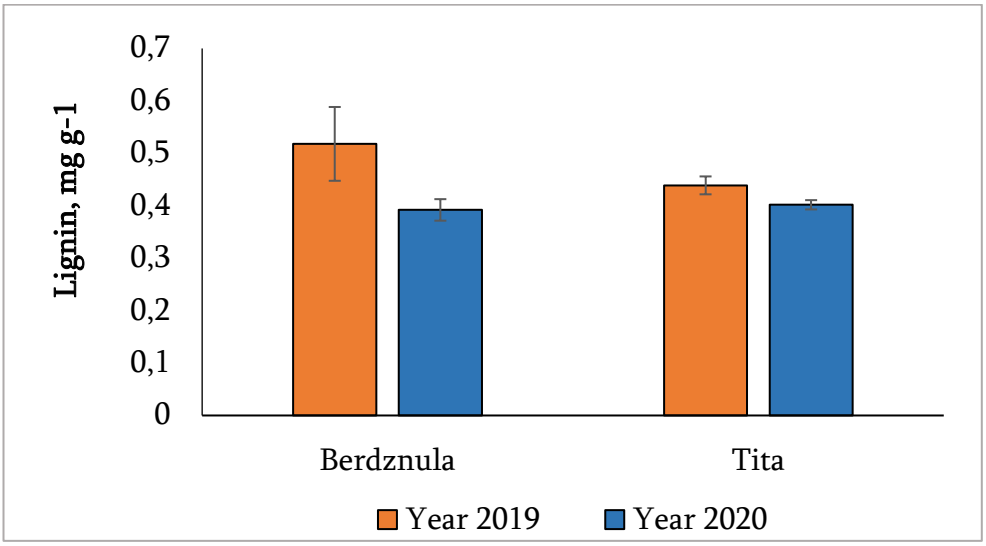


Fig. 14. Lignin content in the nutshell of different hazelnut cultivars in 2019-2020 from II category

Tita begins to accumulate lignin later than Berdznula (Fig. 13). Analogical results were obtained according to kernel damage percentage in 2019-2020 (Fig. 10). In particular, during

both years Tita showed higher levels of damage by *H. halys*. In 2019, 46.7% of the fruit of Berdznula and 63% of the Tita were damaged. In 2020, both the number of *H. halys and* damaged nuts decreased, but the damage in Tita (53%) was more severe than in Berdznula (10.6%) (Fig. 10).

4.10 Changes in length of *H. halys* stylet according to the stage

Stylet length of *H. halys* differs significantly between developmental stages (one-way ANOVA, F = 367.054, df = 2, P < 0.05 followed by Tukey HSD test) In the second instar, the stylet length of *H. halys* is approximately 2 mm, it's the time when it begins feeding with plant tissues, the stylet grows up and reaches 7-8 mm at the adult stage (Fig. 15).

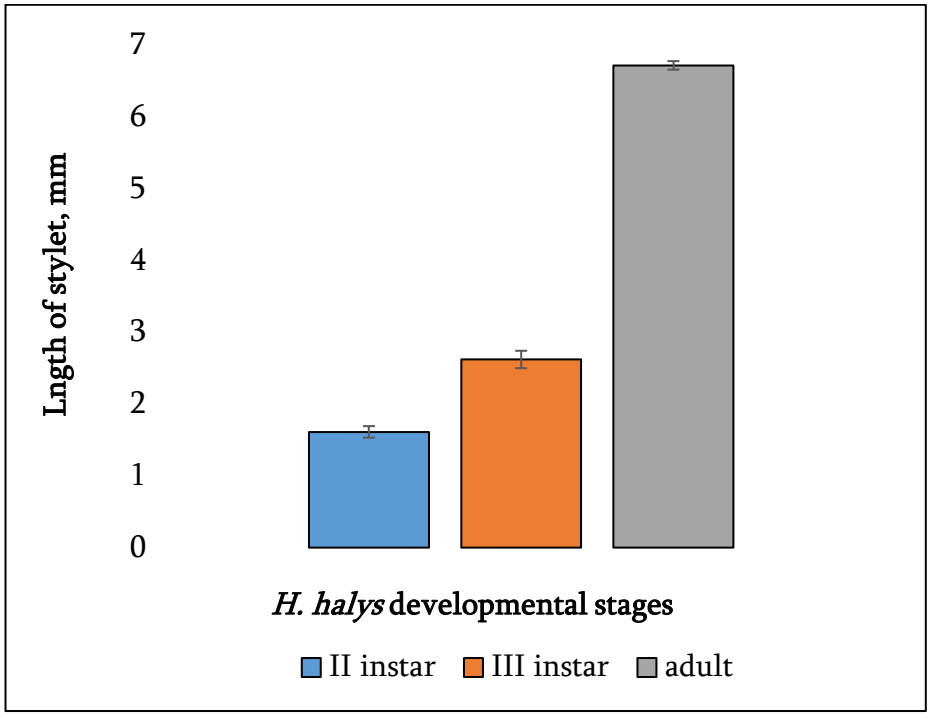


Fig. 15. Length of *H. halys* stylets of different nymph stages

4.11 Natural enemies

In the population of *H. halys* seven natural enemies were identified (Table 5).

Table 5. Natural enemies of *H. halys*

Predators (order)	**Species**
Mantidae	*Hierodula transcaucasica*
	Iris polistictica
Reduviidae	*Rhynocoris iracundus*
Araneae	Different species
Coleoptera	Dermeste sp.
Entomopathogens	**Species**
Hypocreales	*Isaria fumosorosea*
Hypocreales	*Beauveria bassiana*

4.12 The Mantis species

Two species of praying mantis were studied more extensively. Four adult individuals of the mantis were obtained in different parts of Georgia (Table 6).

Table 6. Mantis species collection sights

Name of insect	**Place of collection**	**GPS**		**Altitude (m)**	**Habitat**
		N/L	E/L		
Hierodula transcaucasica	Guria	42° 2' 9.9528''	41°56'21.1956''	70	Hazelnut, citrus orchards

Hierodula transcaucasica	Tbilisi	41°48'19.5192''	44°46'6.6''	446	Nature, Dendropark
Hierodula transcaucasica	Tbilisi	41°48'19.5192''	44°46'6.6''	446	Nature, Dendropark
Iris polystictica	Tbilisi	41° 43' 43.194''	44°51'21.9924''	606	Nature, Dendropark

According to the morphometric analysis, the dimensions and shapes of the three specimens were almost identical, the similarities of which were confirmed by One-way ANOVA analysis with low standard deviation and standard error rates (P-value ≤ 0.05) (Table 7).

Table 7. Results of statistical analyses of morphometric data of *H. transcaucasica* in mm.TL = Total length, HL =Head length, HW = Head width, LFL =Lower frons length, LFw=Lower frons width,PL = Protonum length, PW = Protonum width, FTi = Front tibia, FTa = Front Taurus, FF = Front femur, FC =Fronc coxa, FWL = Front winglength, FWW = Front wingwidth, HWL = Hind winglength, HWW = Hind wingwidth.

Groups	*Count*	*Min*	*Max*	*Sum*	*Average*	*Variance*	*St. dev.*	*St. error*
TL	3	65	72	204	68	13	2.94392	1.69967
HL	3	4.10	5.20	13.8	4.6	0.31	0.55678	0.32146
HW	3	6.50	7.20	20.4	6.8	0.13	0.36056	0.20817
LFL	3	1.90	2.10	6	2	0.01	0.10000	0.05774
LFW	3	2.10	2.30	6.7	2.23	0.01	0.11547	0.06667
PL	3	16.00	19.00	53	17.67	2.33	1.52753	0.88192
PW	3	6.30	7.60	21.1	7.03	0.44	0.66583	0.38442
FTi	3	5.40	5.90	17.1	5.7	0.07	0.26458	0.15275
FF	3	12.10	14.20	39.6	13.2	1.11	1.05357	0.60828
FC	3	10.50	11.60	32.9	10.97	0.32	0.56862	0.32830

FWL	3	41.00	43.00	126	42	1	1.00000	0.57735
FWW	3	12.00	13.00	38	12.67	0.33	0.57735	0.33333
HWL	3	36.00	37.00	110	36.67	0.33	0.57735	0.33333
HWW	3	2.00	2.30	6.5	2.17	0.02	0.15275	0.08819

P-value2.05E-32. TL (Total Length) was used as a factor.

limited number of individual of *Iris polystictica* have been found, wich was drastically different in shape and size, where: Total length - 35mm, Head length - 3.9 mm, Head width - 4.1 mm, Lower frons length - 1.4 mm, Lower frons width - 2.4 mm, Protonum length - 5.4 mm, Protonum width - 1.6 mm, Front tibia 2.9 mm, Front Taurus - NI, Front femur - 1.6 mm, Fronc coxa - 6.3 mm, Front wing length - 13 mm, Front wing width -5 mm, Hind wing length - 10 mm, Hind wing width - 8 mm. Also, this specimen's lower wing was sharply contrastingly painted which was visually easily noticeable.

Based on morphometric analysis and morphological observation confirmed the probable species affiliation of the individuals. From four specimens, three belong to *Hierodula transcaucasica* and one of them - *Iris polystictica* (Fig. 16), because their proportions correspond to the values established by Battison and Massa (2008). We also compared the identified specimens with the data of other researchers (Patel, S., & Singh, R. 2016, Romanowski et al., 2019) which confirmed the accuracy of the species affiliation of the individuals described.

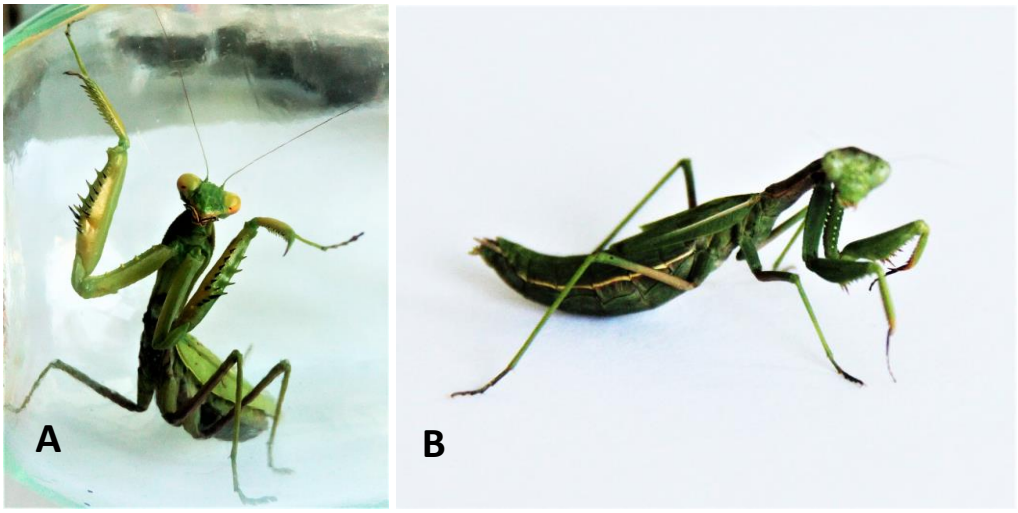


Fig. 16. A - *Hierodula transcaucasica;* **B** - *Iris polystictica*

According to Romanowski (2019, the pronotum of *Hierodula transcaucasica* is short with expanded edges and general shape rounded or ovoidal, without any evident narrowing before the supracoxal dilatation (Figure 17.A, B). Inner side of the fore coxae without large yellowish spots at the base of the marginal spines. *H. transcaucasica's* frontal sclerite (FS) is almost as high as broad (Figure 18 A.B). Knee of the mid and hind femora (HF) with a small spine (Figure 19.B, C, D). Medial face of the fore coxae (FC) without a black spot (Figure 16.A) (Battiston R. & Massa B. 2008).

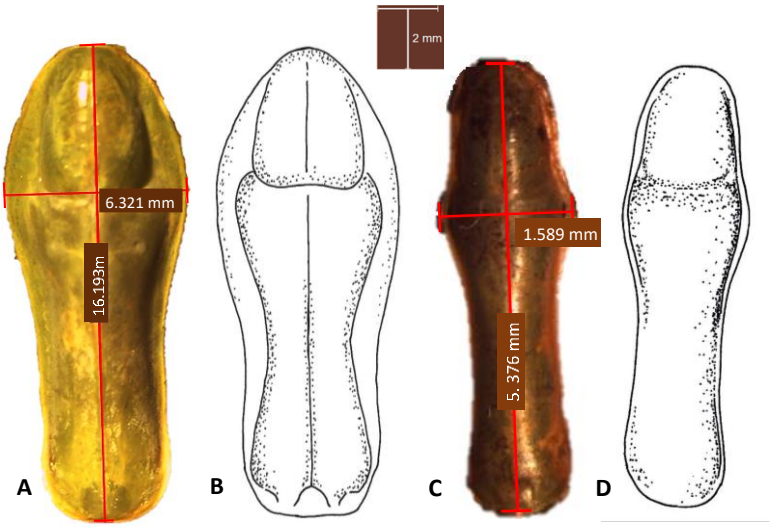


Fig. 17. Pronotum top view under microscope: A- *H. transcaucasica*; C - *I. polystictica;* Drawing of pronotum: B- *H. transcaucasica; D- I. polystictica*

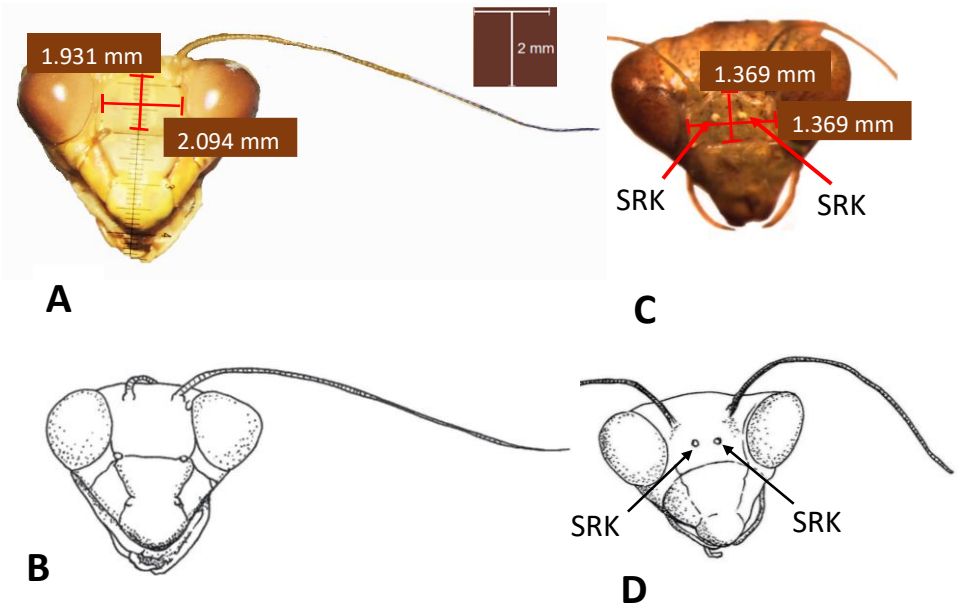


Fig. 18. A –*H.transcaucasica* frontal sclerite highness and broadness; **B** – drawing of *H. transcaucasica* head; **C** - remarkable signs on the frontal sclerite (FS) *Iris polystictica* 2 small round knobs (SRK); **D** - drawing of *I. polystictica* head

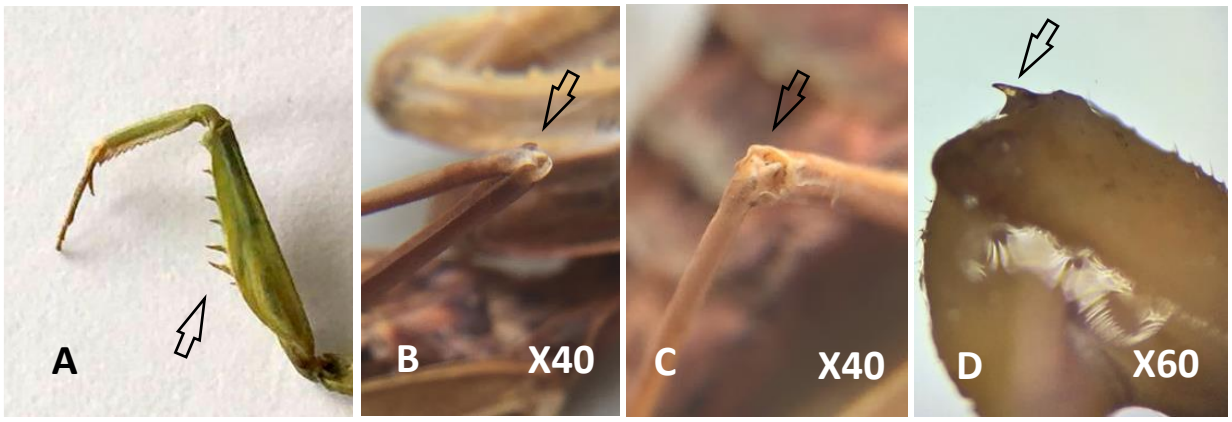


Fig. 19. A .- *I. polystictica* femora with 5 external spines; **B** – *H. transcaucasica*knee of the mid femora with a remarkablesmall spine, **C** – *H. transcaucasica*knee of the hind femora with a remarkablesmall spine. **D** - *H. transcaucasica* spine

The presence of *Iris polystictica* was observed in the examined samples with known remarkable signs: fore femora (FF) with 5 external spines (Figure 19.A) . Frontal sclerite (FS)

of *Iris polystictica* with 2 small round knobs (SRK) (Fugure 18.C, D) (Battiston R. & Massa B. 2008).

4.13 Predation of the mantis

In laboratory experiments predation of adult *H. transcaucasica* on adult *H. halys* were confirmed (Figure 20, A, B). After two-day starvation *H. transcaucasica* prayed seven adult of *H. halys*, next days average number of predations were three exemplars during one day (Fig. 21).

In order to confirm the predation of the *H. transcaucasica* nymph on *H. halys* under natural conditions, conducted the following experiment: *H. transcaucasicas* nymphs (age III), hatched in the laboratory transferred in nature.

In semi-field experiment, three-day starving of one *H. transcaucasica* and 28 newly hatched *H. halys* nymphs (on the branch of *Rubinia pseudoacacia*) under the one cage were satellite (Figure 20, C).

On the first hour, *H. transcaucasica* predate on seven nymphs of *H. halys*. Average number of destroyed newly hatched nymphs during one hour, three exemplars were amounted (Fig. 22).

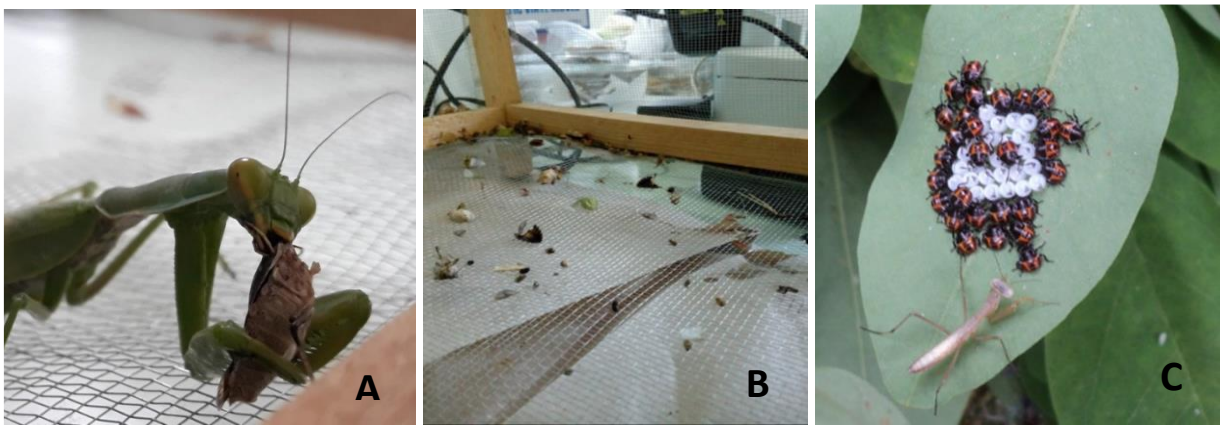


Fig. 20. A - predation of adult *H. transcaucasia* on *H. halys* imago; **B** - residues after feeding; **C** - *H. Transcaucasia* nymph predation *H. halys* on newly hatched nymphs

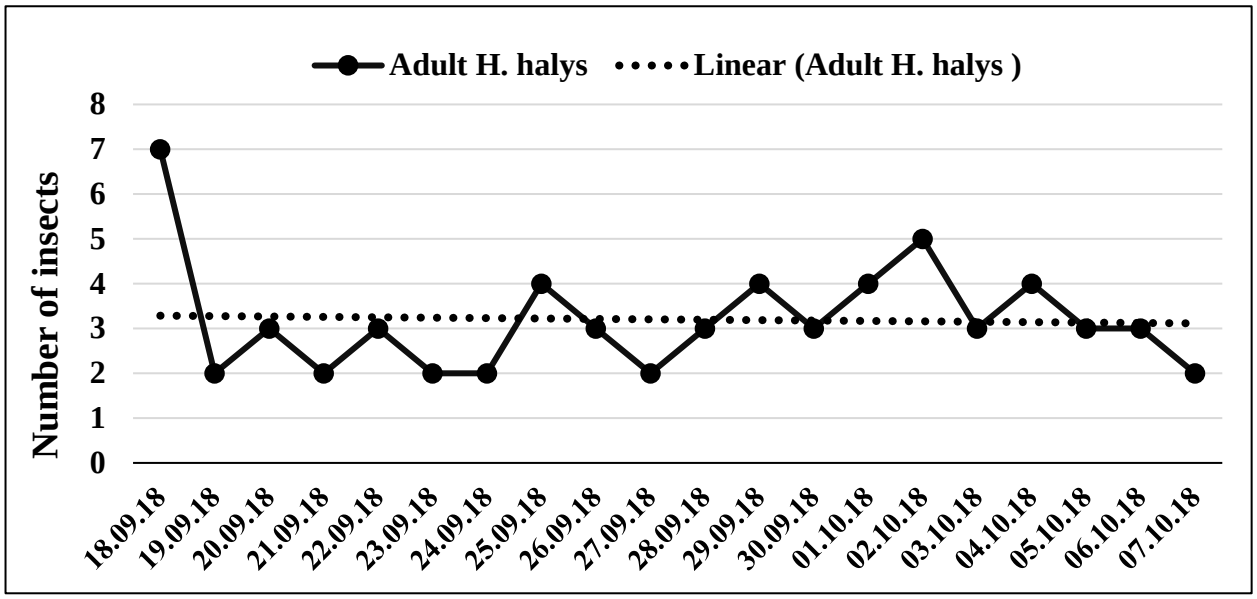


Fig. 21. Adult *H. transcaucasica* predation in adult *H. halys* adult individuals in the laboratory

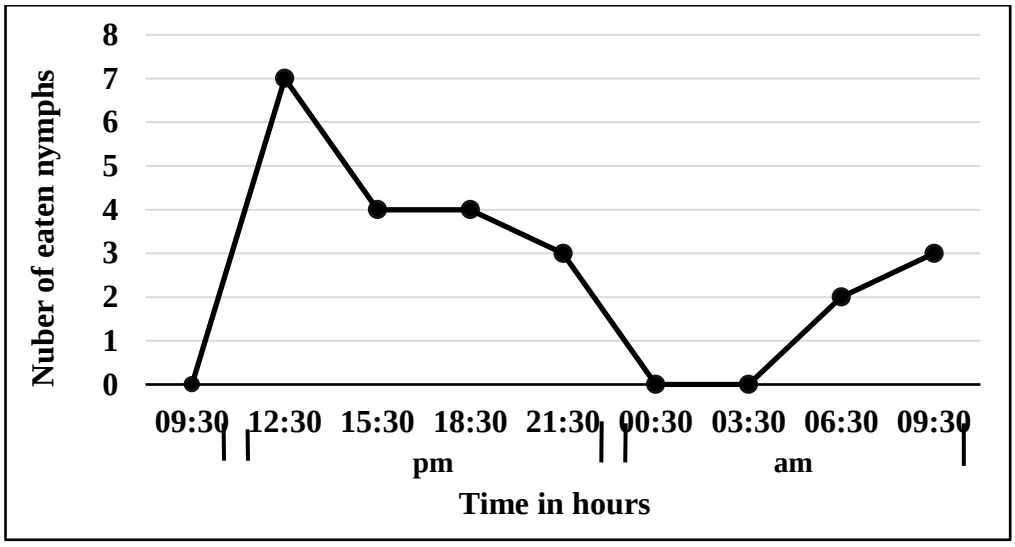


Fig. 22. *H. transcaucasicas* nymph predation of *H. halys* on newly hatched nymphs in the laboratory for 24 hours

Nymph predation of *H. transcaucasicas* was confirmed on *H. halys* nymphs of age I (Figure 20, C). As can be seen from Figure 22, one hungry individual killed 23 (83%) newly hatched nymphs of *H. halys* in 24 hours. It was observed that the nymph of *H. transcaucasicas* was not fed during the period 0:30-3:30 am.

The daily and hourly feeding intensity of *H. transcaucasica* was statistically checked (Fig.23).

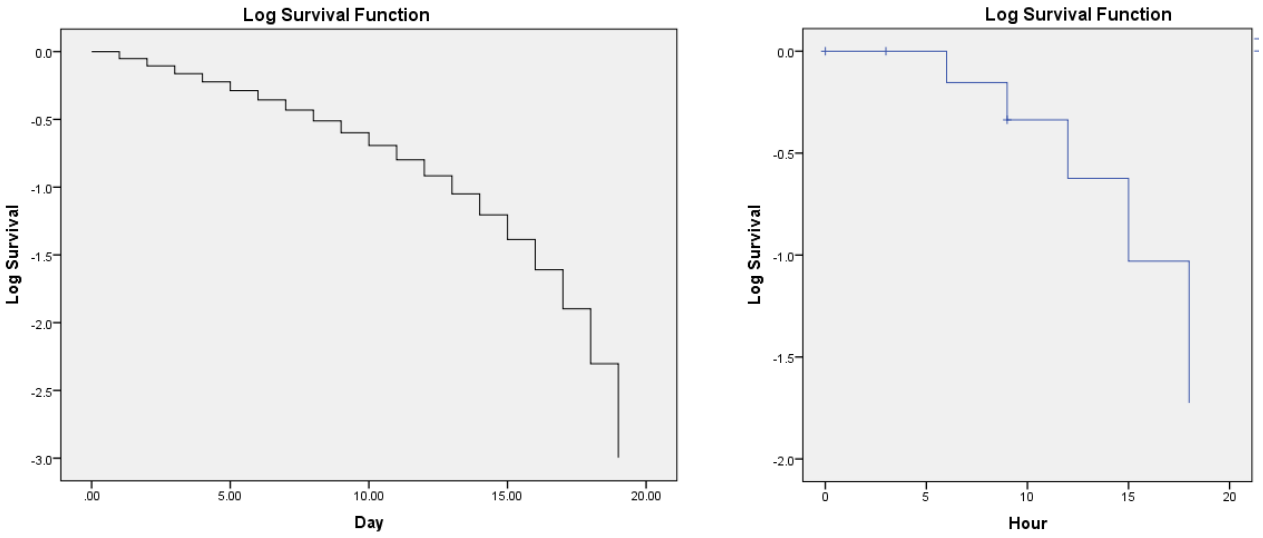


Fig. 23. Kaplan-Meier survival plots for *H. transcaucasica* adults. **A**- shows the Kaplan-Meier survival curves of adult *H. halys* during 20 days of exposure with adult *H. transcaucasica*; **B** - shows survival curves of nymph *H. halys* during 24 hours exposure with *H. transcaucasica* nymphs

According to chi-square test ($\chi 2$=80.00, P=0.355) no significant correlation were found between adult *H. halys* mortality and quantity of the experimental days. Also, no significant correlation ($\chi 2$= 32,250, P= 0.264) were found between mortality of *H. haly* nymphs and duration in hours.

Descriptive statistics mean and median confidence intervals for survival time (adult and nymph *H. halys*) is given in table 8. The median for the Adult *H. transcaucasica* was 10 days, and for the *H. transcaucasica* nymph was 15 hours, meaning that 50% of the population of *H. halys* were destroyed during this time.

Table 8. Mean and median for survival time

Predator	Mean[a]				Median			
	95% Confidence Interval							
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
Adult	10.500	1.323	7.907	13.093	10.000	2.236	5.617	14.383
Nymph	13.929	2.160	9.695	18.162	15.000	3.325	8.483	21.517

a. Estimation is limited to the largest survival time if it is censored.

4.14 Efficacy of entomopathogenic nematodes against *H. halys*

All the four tested isolates *H. bacteriophora* HRB (GEO), *S. borjomiense*, HRB (IT) and *S. apuliae*, were able to infect the adults of *H. halys* and efficiency of IJs varied depending on the EPNs strains. Experimental data showed that all entomopathogenic nematodes tested were able to kill *H. halys* (Fig. 24). Significant differences were observed between Georgian and Italian strains. At the end of experiment, cumulative mortality for HRB (GEO) and *S. borjomiense* at high concentration (1000 IJs/adult) reached 53.3% and 40%, respectively, whereas at concentrations of 500 and 200 IJs/adult the mortality ranged 40–33.3% and 33.3–13.3%, respectively. For the Italian strains HRB (IT) and *S. apuliae*, the recorded mortality at high, intermediate and low concentrations ranged between 95.3–60%, 93.3–40% and 73.3–33.2%, respectively. The emerging IJs were harvested and counted throughout the interval of 8–10 days after treatment. All dead BMSB specimens showed symptoms of infection with nematodes developing and reproducing into adults (Fig. 25).

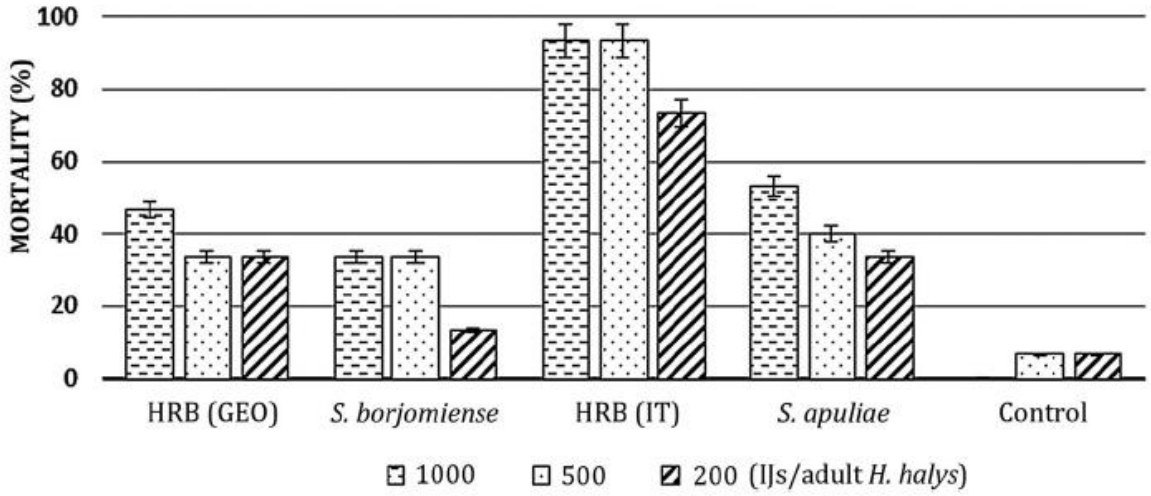


Fig. 24. Comparative efficiency of different strains of the entomopathogenic nematodes *H. bacteriophora* (GEO) (IT), *S. borjomiense* HRB (GEO) and *S. Apuliae* (IT), against adults of *H. halys*, in laboratory assay.

Suppressive effectiveness of all nematodes enhanced as the exposure period increased. The largest increase, between days four and eight, was noticed between *H. bacteriophora* (IT) and *S. apuliae* at the concentration of 1000 IJs/adult where effectiveness in BMSB suppression of the first strain was about twice as much as in the second. A mortality rate of 6.66% was recorded among control insects. Experimental data are presented in Figure 26. On the 3rd day post-treatment, *H. halys* adults mortality at the highest concentration showed a 6.7% variant only for *H. bacteriophora* (IT). The same mortality rates (6.7%) were displayed in variant *H. bacteriophora* (GEO), *S. apuliae* and *S. borjomiense*, but on the 4th day. Maximum mortality was recorded after 8 days by *H. bacteriophora* (IT) with 95.5%, *S. apuliae* with 60%, *H. bacteriophora* (GEO) with 53.3%, and *S. borjomiense* with 40% (Figure 26(A)). At the concentration of 500 IJs/adult, mortality in the variant of HRB (IT) was 6.7% on day 3 and 93.3% on day 8. Four days are needed for the nematodes to kill 6.7% insects for the variant of *S. apuliae*, whereas 5 days are needed for HRB (GEO) and *S.borjomiense* to kill the same number of insects. After 8 days post-treatment, *S. apuliae* and *H. bacteriophora* (GEO) reached 40% mortality of BMSB, while *S. borjomiense* reached 33.4% mortality (Figure 26(B)).

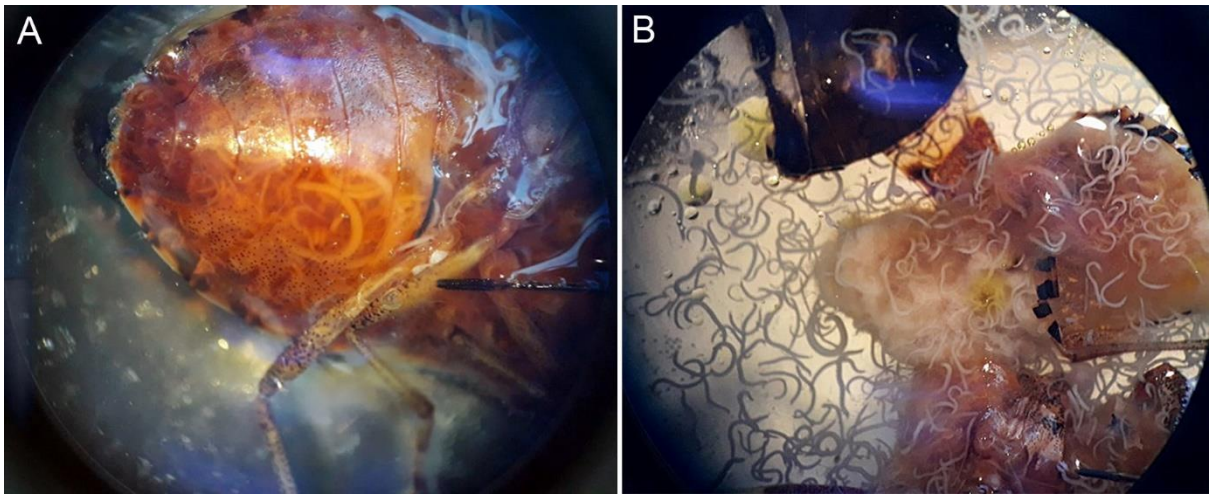


Fig. 25. Adults of *H. halys* infected by *Heterorhabditis bacteriophora* (Italian strain), life stages, inside the body (A), and released outside (B) after dissection of insect body.

Low rate of mortality was observed at concentration of 200 IJs/adult (Figure 26(C)). With the exception of *H. bacteriophora* (IT), that killed 6.7% and 73.4% of tested insects at days 4 and 8, respectively; *S. apuliae*, *H. bacteriophora* (GEO) and *S. borjomiense* reached a

mortality of 6.7% at the 5th day and of 33.3%, 33.4, 13.4%, respectively, at the 8th day post infection. A change in colour of nematode-invaded insects was usually observed, with insects turning dark yellow-brown (Poinar, 1976) when infected by *H. bacteriophora* and darker, or grey to dark-grey when infected by *Steinerma* spp. The susceptibility of *H. halys* to a particular nematode species and concentration is a key factor for LC50 levels. The concentration required to cause 50% mortality of *H. halys* for different strains of EPNs, namely *H. bacteriophora* (GEO), S. borjomiense, *H. bacteriophora* (IT) and *S. apuliae*, are presented in Table 8.

All four strains are pathogenic at the highest concentration (1000 IJs/adult), with a mean mortality ranging from 40 to 95.5% ($P \leq 0.05$). The highest percentage of mortality was observed 8 days after treatment. *H. bacteriophora* (IT) was significantly more virulent than the other strains at all tested concentrations. Probit analysis used to analyse the mortality results revealed that *H. bacteriophora* (IT) had the lowest median lethal concentration value, whereas S. *borjomiense* had the highest (Table 9).

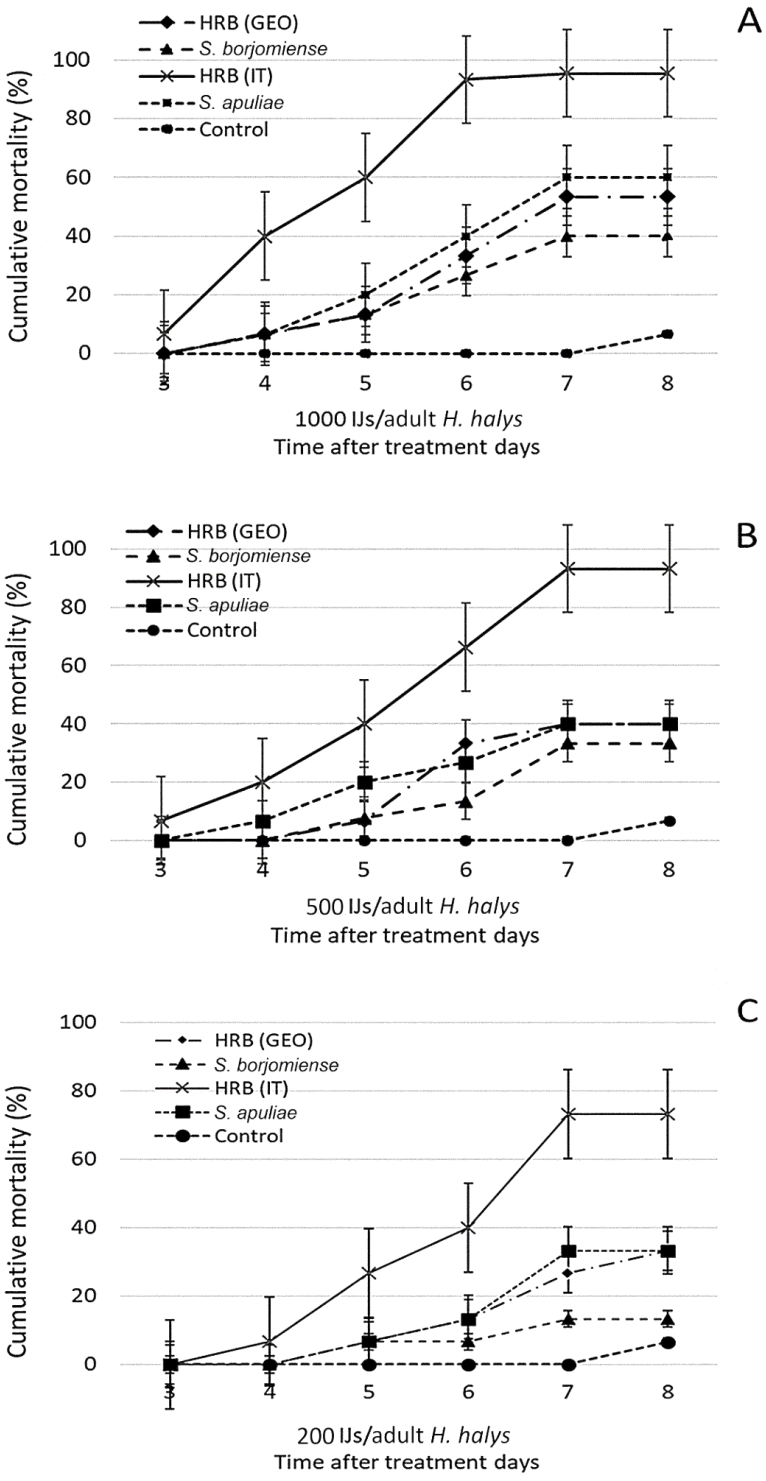


Fig. 26. Cumulative mortality of adult of *H. halys* by *H. bacteriophora* (GEO), *S. borjomiense*, *H. bacteriophora* (IT) and *S. apuliae* with different concentrations (A – 1000, B – 500, C – 200 IJs/adult) according to days after treatment. Dashed lines indicate mortality in controls. Vertical bars indicate standard error.

Table 9. Median lethal concentration LC50 (IJs/ml) of *H. halys* adult treated by the four EPN strains (ANOVA, P ≤ 0.05).

Strain	Slope±SE	LC%50 PPM	95% Fiducial CI		Chi-test (χ2) Sig	df	*P-value*
			Lower	Upper			
HBR (GEO)	0.721±0.322	879.289	205.395	3764.204	0.871	1	0.015
S. borjomiense	1.252±0.199	1390.821	567.433	3409.005	0.748	1	0.028
HBR (IT)	1.255±0.228	64.171	22.930	179.589	0.997	1	0.034
S. apuliae	0.951±0.245	656.671	217.570	1981.965	0.882	1	0.039

The concentration required to cause 50% mortality of *H. halys* for the four tested strains of EPNs at high, medium and low concentrations is presented in Figure 27 (A–D). One-way ANOVA analysis shows that the average mortality was significantly (P ≤ 0.05) affected by exposure of *H. halys* insects to different concentrations of EPN suspensions. Insects exposed during the period 3–8 days post-infection (dpi) at the highest concentration (1000 IJs/adult), had significantly increased death rate. The lethal survival time (LD50) of *H. halys* (Fig. 28) ranged from a minimum of 4.3 to a maximum of 6.6 days (Table 10). Survival curves for treatments with a concentration of 1000 IJs/adult, all differed based on the Kaplan–Meier method. Pearson's Chi-square statistic test (all values of P > 0.05) indicated that the data fitted the regression models according to Breslow (Generalised Wilcoxon). Time-Response Bioassay Survival analysis of *H. hayls* adults exposed to the control and the selected nematodes with concentration 500 and 200 IJs/adult indicated no significant difference between the mortality times (Figures 26 and 27). Survival curves for treatments with concentration 500 IJs/adult (Fig. 29) Pearson's Chi-square statistic test (all values of P > 0.05) indicated that the data fitted the regression models, where x2= 0.292, df = 1, sig = 0.589 and with the concentration of 200 IJs/adult (Figure 30) x2 = 0.839, df = 1, sig = 0.360 according to Breslow (Generalised Wilcoxon). Survival curves for all treatments differed by the Holm–Sidak method (P < 0.05).

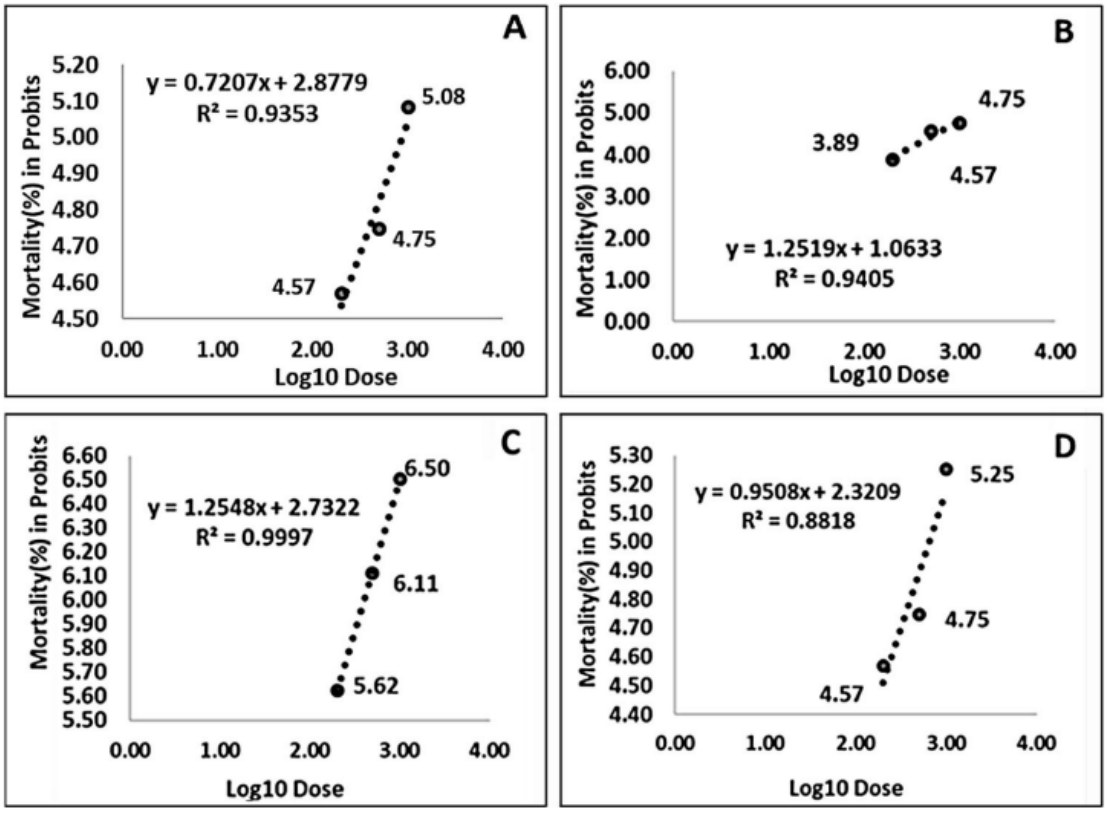


Fig. 27. Probit mortality of *H. halys* adults exposed to different concentration: (A) HRB (GEO), (B) *S. borjomiense*, (C) HRB (IT), (D) *S. apuliae*.

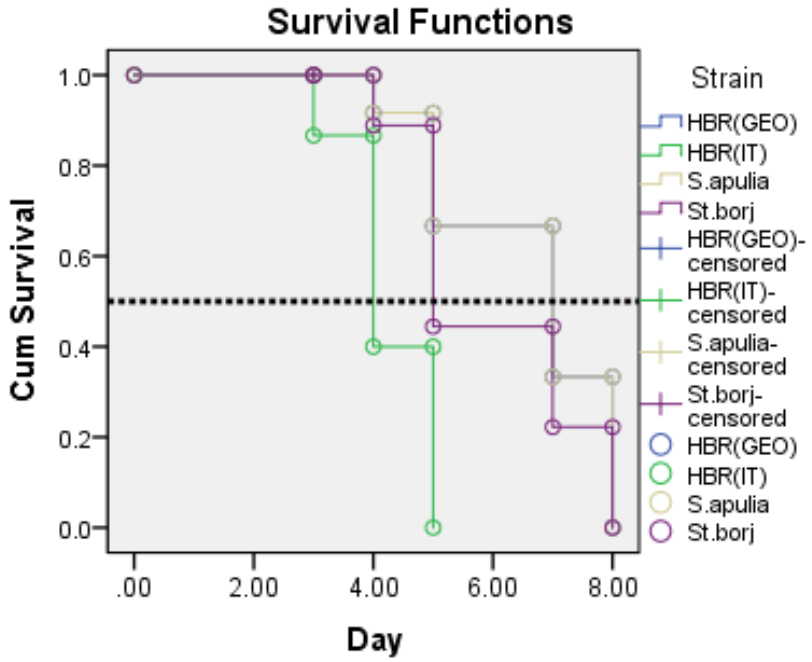


Fig. 28. Survival curves (Lethal time LD50) of *H. halys* adults treated by nematodes suspension of 1000 IJs/adult.

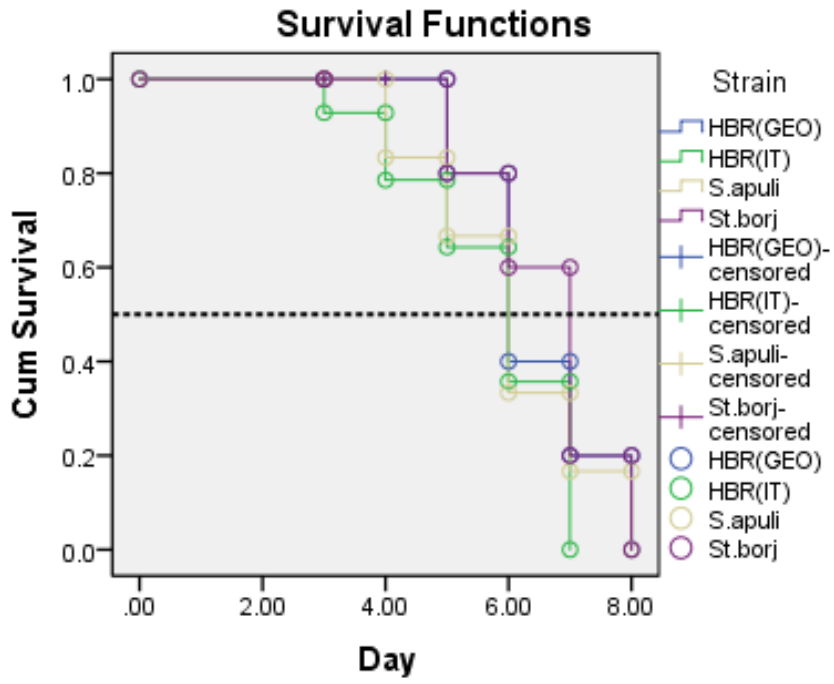


Fig. 29. Survival curves (Lethal time LD50) of *H. halys* adults treated by nematodes suspension of 500 IJs/adult.

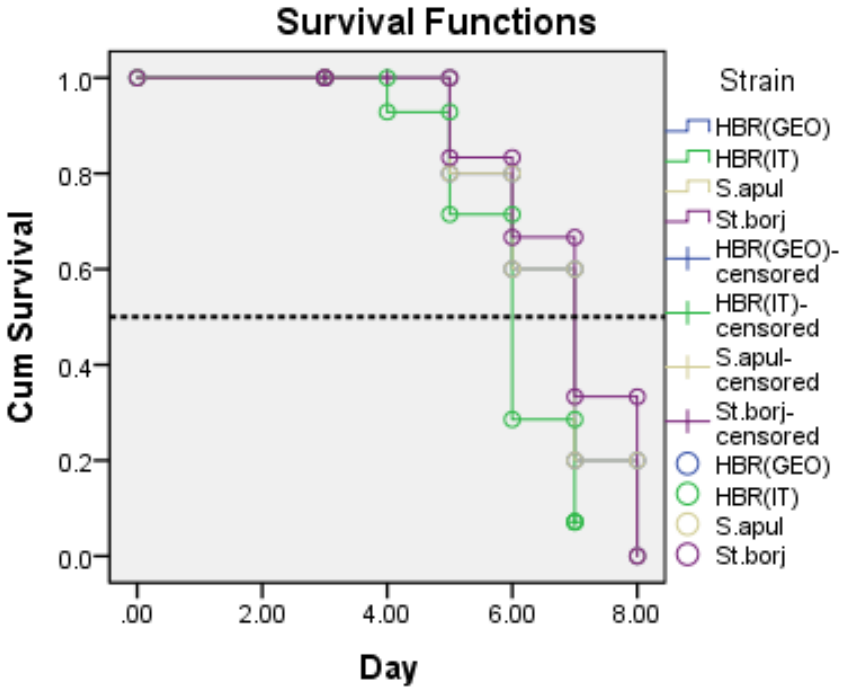


Fig. 30. Survival curves (Lethal time LD50) of *H. halys* adults treated by nematodes suspension of 200 IJs/adult.

Table 10. Mortality (%), mean survival time and LT50 (days) of *H. halys* adult-treated nematode strains with concentration 1000 IJs/adult.

Name of Nematodes	Mortality %[a]	Mean survival time ± SE[b]	LT50 (95% CI)	N[c]
HRB (GEO)	53.3	6.583±0.417	7.000	15
St.Borjomiensis	40.0	6.000±0.500	5.000	15
HRB (IT)	93.3	4.267±0.182	4.000	15
S.apuliae	60.0	6.583±0.417	7.000	15

[a]Percent of dead individuals at the end of experiment corrected for mortality in control using Abbott's formula;
[b]The mean survival time and its standard error were underestimated because the largest observation was censored;
[c]Total number of insects in bioassay.

Table 11. Mortality (%), mean survival time and LT50 (days) of *H. halys* adult-treated nematode strains with concentration 500 IJs/adult.

Name of Nematodes	Mortality%[a]	Mean survival time ± SE[b]	LT50 (95% CI)	N[c]
HRB (GEO)	33.3	6.400±0.510	6.000	15
St.Borjomiensis	33.3	6.600±0.510	7.000	15
HRB (IT)	93.3	5.714±0.354	6.000	15
S.apuliae	40.0	6.000±0.577	6.000	15

Table 12. Mortality (%), mean survival time and LT50 (days) of *H. halys* adult-treated nematodes strains with concentration 200 IJs/adult.

Name of Nematodes	Concentration	Mortality%[a]	Mean survival time ± SE[b]	LT50 (95% CI)	N[c]
HRB (GEO)	200	33.3	6.600±0.510	7.000	15
St.Borjomiensis	200	13.3	6.833±0.477	7.000	15
HRB (IT)	200	73.3	5.929±0.246	6.000	15
S.apuliae	200	33.3	6.600±0.510	7.000	15

4.15 Potential of Bover-Ge

Bover-Ge was tested in the laboratory under 1 x 10^7 and 1X 10^8concentrations against adult of *H. halys*. At the 1X10^7 concentration effectiveness was about 70% and 1X 10^8concentration effectiveness reached about 90% (Fig. 31).

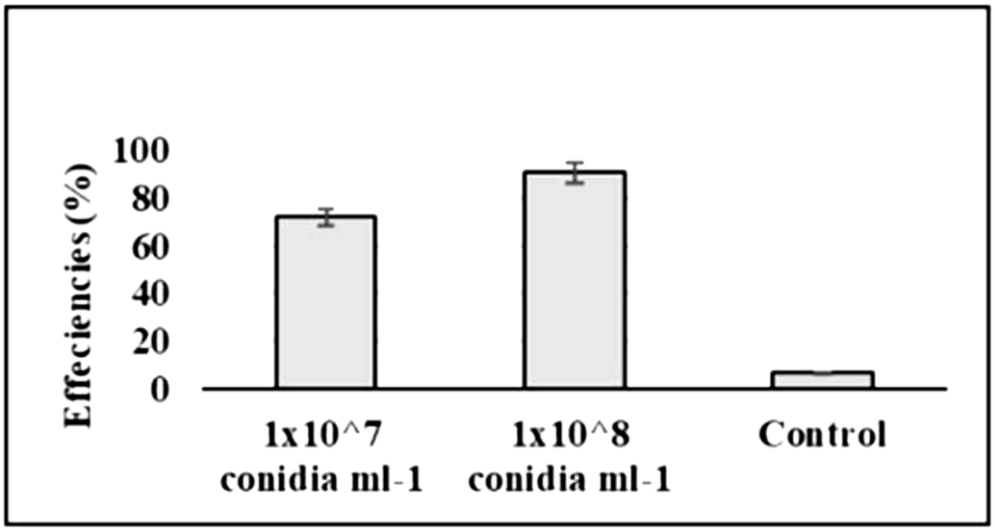


Fig. 31. Efficiency of Bover-Ge on the adult *H. halys* under laboratory conditions

Bover-Ge was tested against overwintering forms of *H. halys*, mortality achives 64.5% (Fig. 32).

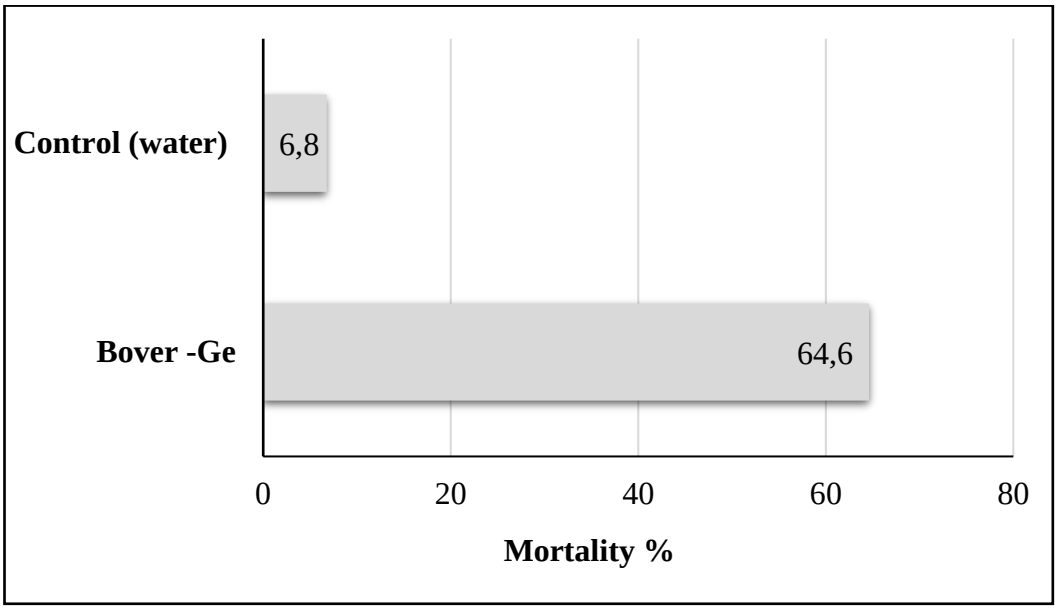


Fig. 32. Efficiency of Bover-Ge on the overwintering *H. halys* in semi-natural conditions.

4.16 Entomopathogenic Fungi isolated from *H. halys* populations

4.16.1 Morphology of isolated fungi

Three isolates (MB 101, MB 102, MB 104) showed white to cream colonies with irregular edges and powdery appearance, typical macroscopic traits of *Beauveria* genus. The microscopic observations of the three strains presented reproductive structures and conidia with typical morphology, size, and color of three *B. bassiana*. Strains showed septate mycelium, conidiogenous cells size of 5.5 to 8.4 µm (SD 0.3 to 0.9 µm) x 2.2 to 2.9 µm (SD 0.2 to 0.6 µm), with a wide base and a narrowed apex from which multiple chained conidia emerge in a rachis with a zig-zag arrangement. The conidia were hyaline and smooth, with a globose to subglobose structure. The diameters of the conidia showed a mean value of 1.6 to 2.4 µm (SD 0.6 to 0.7 µm) (Fig. 33).

Isolate - MB 103 fungal colony grown in PDA media was white to light pink in color and appeared woolly and powdery. Droplets of exudate in a small amount, small, colorless or completely absent. The reverse side of the colonies is white at first, then pale yellow from the dentrum to the periphery. The conidial structures are very simple, consisting of single phialides or whorls of phialides, or of short conidiophores, simple or slightly branched, bearing terminal glomeruli of phialides. Conidiophores 7-15 µm long, 1.5-2 µm in diameter, smooth. Phialides in whorls 5.7-18 µm long with a diameter at the base of 1-2 µm, tapering into a long neck 0.5 µm in diameter. Conidiphores with rounded or pointed ends, 3-4×1-2 µm, in chains, mostly 45-50 µm long, but sometimes up to 90 µm. Conidia elongate before germination and give rise to a polar germ tube (Fig. 33).

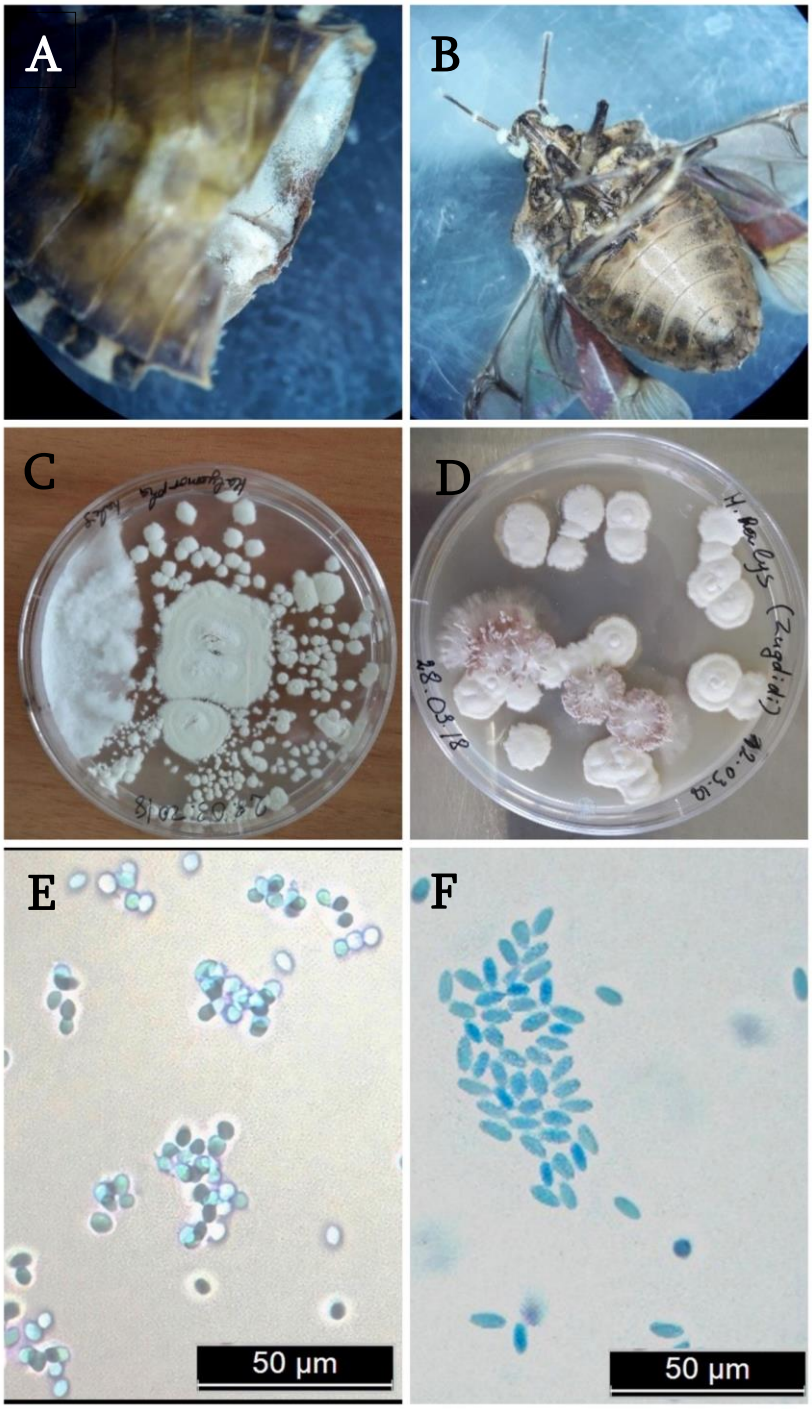


Fig. 33. Isolation and morphological characteristics of the entomopathogenic fungi. A, B- Symptoms of the fungal infection of *H. halys* under the microscope, colony growth on the PDA artificial media C – *Beauveria bassiana*, D- *Isaria fumosorosea*, Spores of the E- *Beauveria bassiana*, F- *Isaria fumosorosea*

4.16.2 Molecular identity

The PCR products of the ITS regions of the rDNA of the all isolated entomopathogenic fungi generated fragments of approximately 600 bp.

The phylogenetic analyses provided sufficient information to complement the morphological data obtained and allowed to classify four fungal strains within the MB101, MB102, MB 104 – *Beauveria bassiana* and MB103 - *Isaria fumosorosea* (Fig 34).

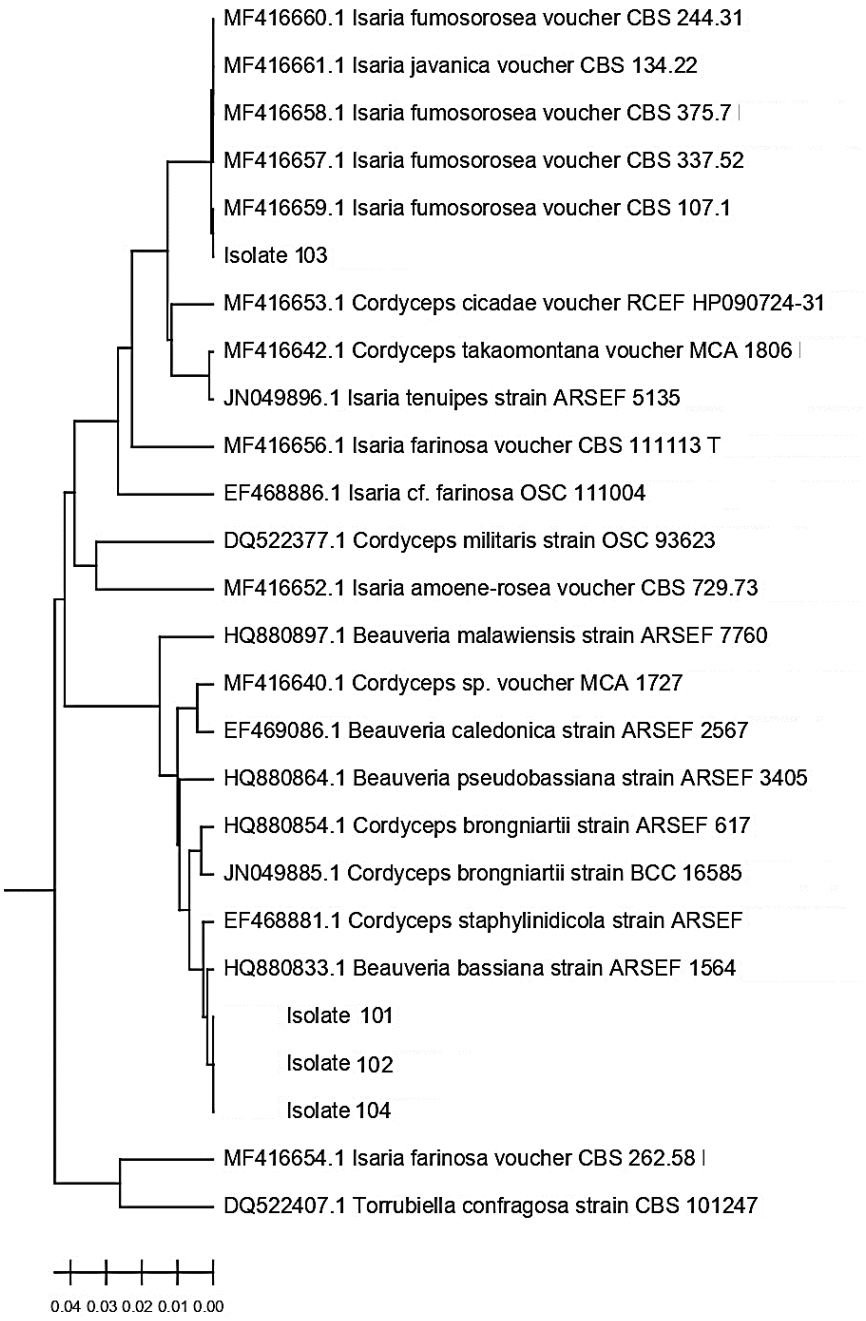


Fig. 34. Evolutionary relationships of taxa

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973). The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura and Kumar, 2004) and are in the units of the number of base substitutions per site. This analysis involved 26 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 644 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura, Stecher and Kumar, 2021).

4.16.3 Enzymatic characteristics of isolated entomopathogenic fungi from *H. halys*

Chitinase activity

The maximum mean chitinase activity (0,6057 U/mL) was recorded in *B. bassiana* MB101 followed by *B. bassiana* MB102 (0,4236 U/mL). In contrast, the minimum mean chitinase activity (0,3398 U/mL) was recorded in *B. bassiana* MB 104 (Table 13). A significant difference was recorded between the chitinase activities on alternative days. The maximum chitinase activity (1,257U/mL) was recorded on the seventh day which's significantly better than that on all other days. Among all days of observations, the minimum mean chitinase activity (0.29 U/mL) was recorded on the second day. The chitinase activity increased from 2nd to 7th day and decreased from 8th to 12th day (Fig. 35)

Table 13. Descriptive statistics of chitinase activity

Isolate	Minimum	Median	Maximum	Mean	Std. Deviation	Std. Error of Mean	95% CI of mean	
							Lower	Upper
MB 101	0,16	0,51	1,257	0,6057	0,3905	0,1177	0,3434	0,868

MB 102	0,01733	0,4197	0,8533	0,4236	0,2841	0,08565	0,2327	0,6144
MB 103	0,0125	0,4403	0,7933	0,4209	0,2365	0,07132	0,262	0,5798
MB 104	0,002	0,2933	0,94	0,3398	0,2677	0,08072	0,16	0,5197

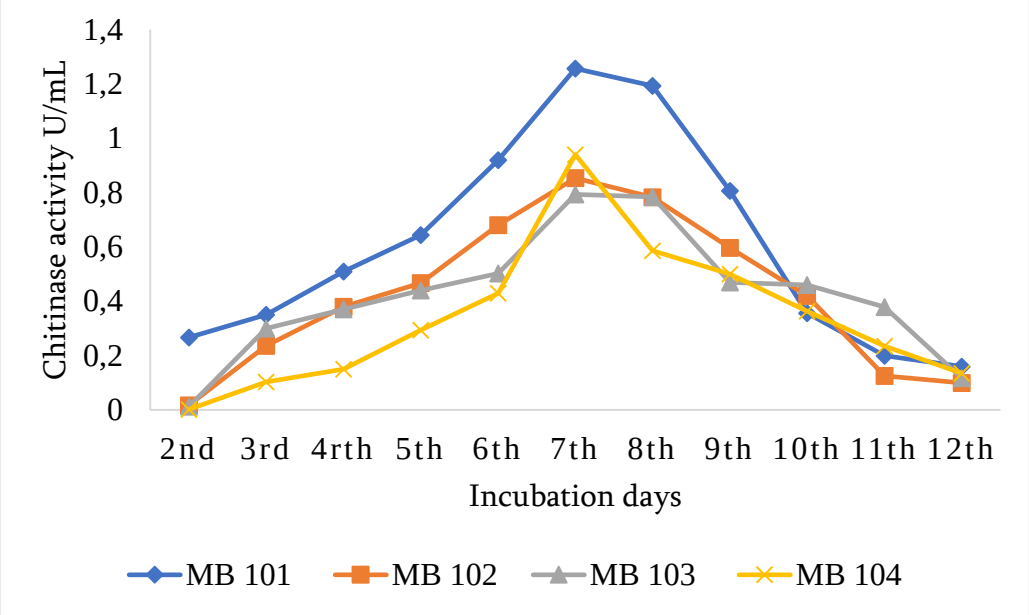


Fig. 35. Chitinase activity of *B. bassiana* and *I. fumosorosea* isolates over time

protease activity

The maximum mean protease activity (1,06 U/mL) was recorded in *B. bassiana* MB 101, followed by *B. bassiana* MB 104 with 0,8892U/mL (Table 14). The minimum mean protease activity (0,7658 U/mL) was recorded in *B. bassiana* MB 102. A significant difference was recorded in the mean protease activities on various days. The maximum protease activity (1.37 U/mL) was recorded on the 8th day of incubation, and the minimum mean protease activity (0.38 U/mL) was recorded MB 103 *Isaria fumosorosea* on the 12th day of incubation. Protease activity increased from day 2nd to 8th, and thereafter, it gradually decreased (Fig.36).

Table 14. Descriptive statistics of protease activity

Isolate	Minimum	Median	Maximum	Mean	Std. Deviation	Std. Error of Mean	95% CI of Mean	
							Lower	Upper
MB102	0,24	0,885	1,06	0,7658	0,2831	0,08172	0,586	0,9457

MB 101	0,55	1,195	1,37	1,06	0,313	0,09035	0,8611	1,259
MB 103	0,38	0,965	1,14	0,8558	0,2799	0,0808	0,678	1,034
MB104	0,38	1,045	1,22	0,8892	0,3286	0,09485	0,6804	1,098

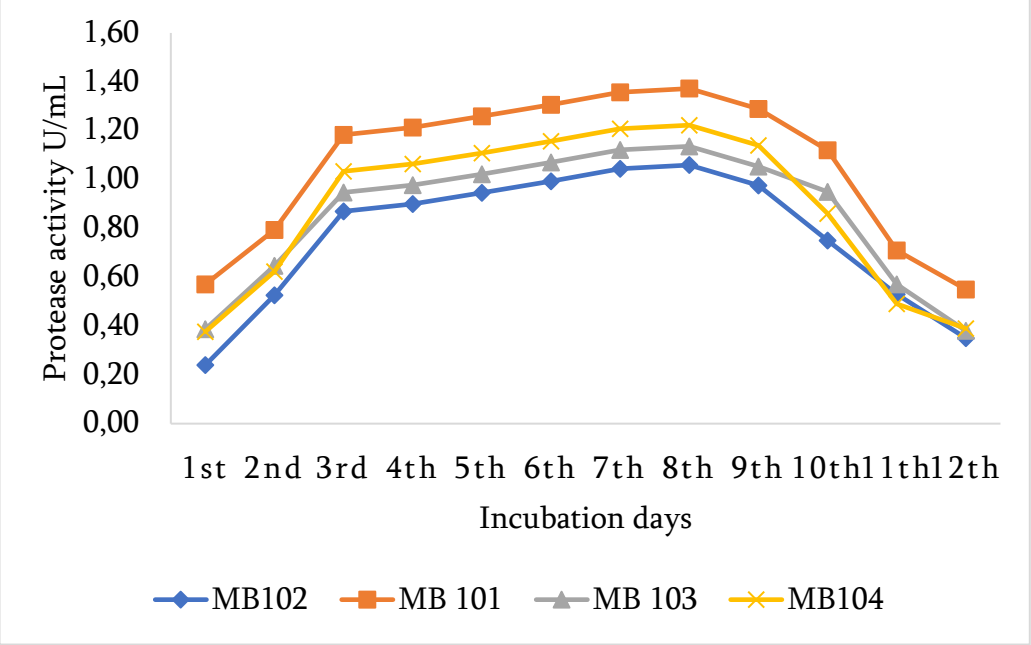


Fig. 36. Protease activity of *B. bassiana* and *I. fumosorosea* isolates according alternative days

Lipase activity

A significant difference was recorded between the mean lipase activities of various isolates of *B. bassiana*. The maximum mean lipase activity (1,564 U/mL) was recorded in *B. bassiana* MB 101 followed by *I. fumosorosea* MB 103 (1,414U/mL). The minimum mean lipase activity (1,306 U/mL) was recorded in *B. bassiana* MB 102 (Table 15). The mean lipase activity of various days was significantly different from each other. The maximum mean lipase activity (2.04 U/mL) was recorded on 6th day, and the minimum mean lipase activity (0.54 U/mL) was recorded on the 2nd day of incubation (Fig. 37).

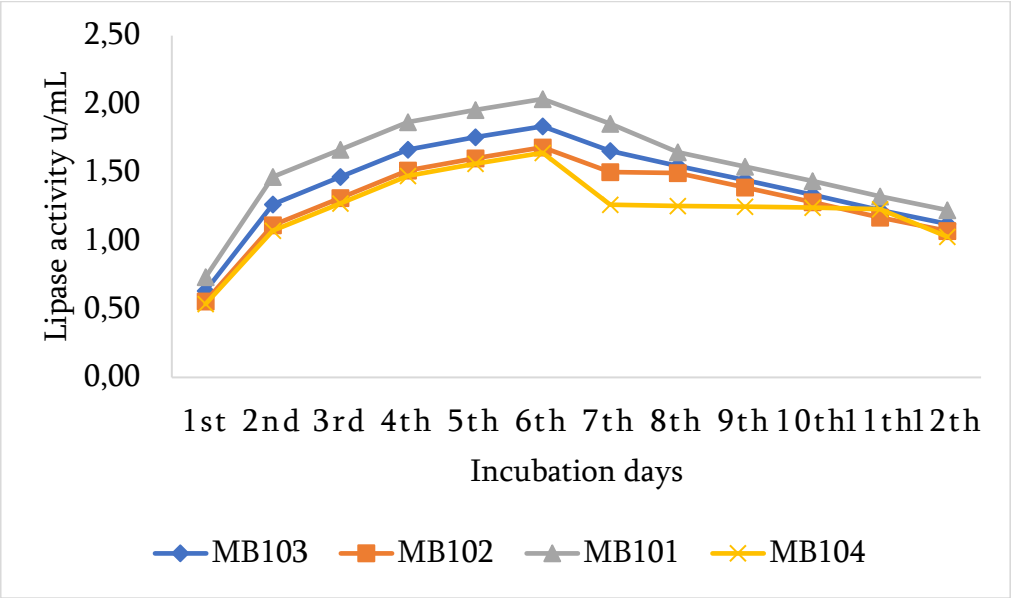


Fig. 37. Lipase activity of *B. bassiana* and *I. fumosorosea* isolates according alternative days

Table 15. Descriptive statistics of lipase activity U/mL

Isolate	Minimum	Median	Maximum	Mean	Std. Deviation	Std. Error of Mean	95% CI Error of Mean	
							Lower	Upper
MB103	0,63	1,455	1,84	1,414	0,3323	0,09592	1,203	1,625
MB102	0,56	1,35	1,68	1,306	0,304	0,08774	1,113	1,499
MB101	0,73	1,595	2,04	1,564	0,367	0,1059	1,331	1,797
MB104	0,54	1,25	1,64	1,234	0,2828	0,08163	1,055	1,414

4.16.4 Dose-Mortality Response

The entomopathogenic fungal isolates were tested at three different concentrations in the laboratory and field conditions to explore their potential to manage the pest population. Per cent mortality of *H. halys* larvae were calculated and showed increasing mortality with increasing spore concentration. Cumulative mortality of *H. halys* over exposure period (3, 5, 7

and 10 days) (Figures 38-44) was significantly (P <0.01) different for fungi isolates (Table 16, 17).

Among the concentrations of entomopathogenic fungi maximum percent mortality was recorded at 1 × 10^8 conidia mL^{-1} of MB 101 (94.04%) followed by MB 10 (76.31%) on 10th day after treatment application. At the highest concentration of conidia, all concentration gave the highest percent mortality (Table 20-22). The results indicated for pathogenicity of all the concentrations revealed that all of them are virulent.

All isolates tested were pathogenic to *H. halys* nymphs. However, the mortality among isolates was variable at different concentrations (Figures 42-44). The MB 104 strain had the lowest measured performance at all concentrations at all evaluation times. No significant differences in mortality were found between the MB 101 and MB 102 strains at the highest concentrations tested (n ˆ 10^7 and n ˆ 10^8) for studies with adults at 10 days after treatment.

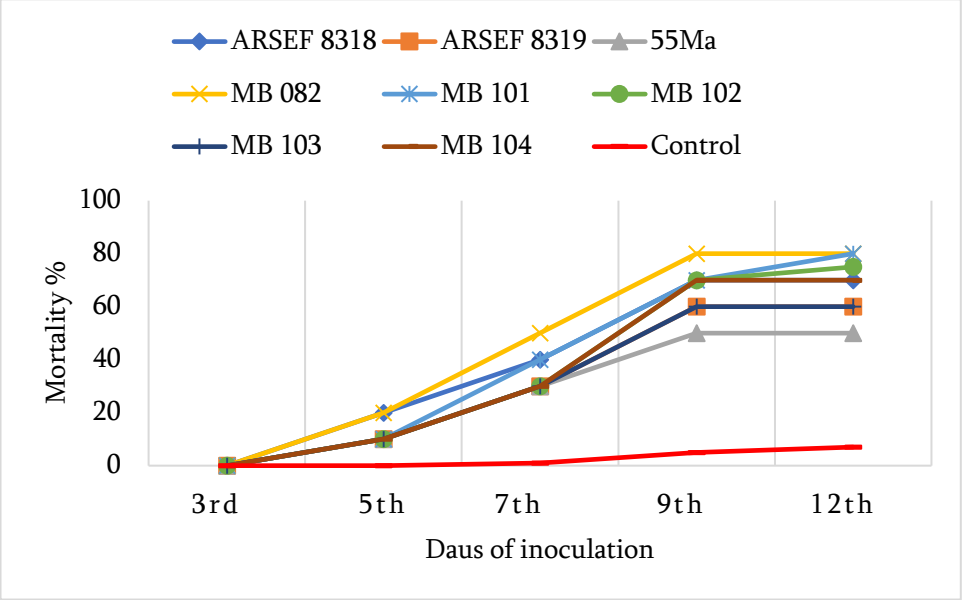


Fig. 38. Cumulative mortality of the local and alien fungal isolates against *H. halys* adults according alternative days, at the concentration 1x10 ^{6}

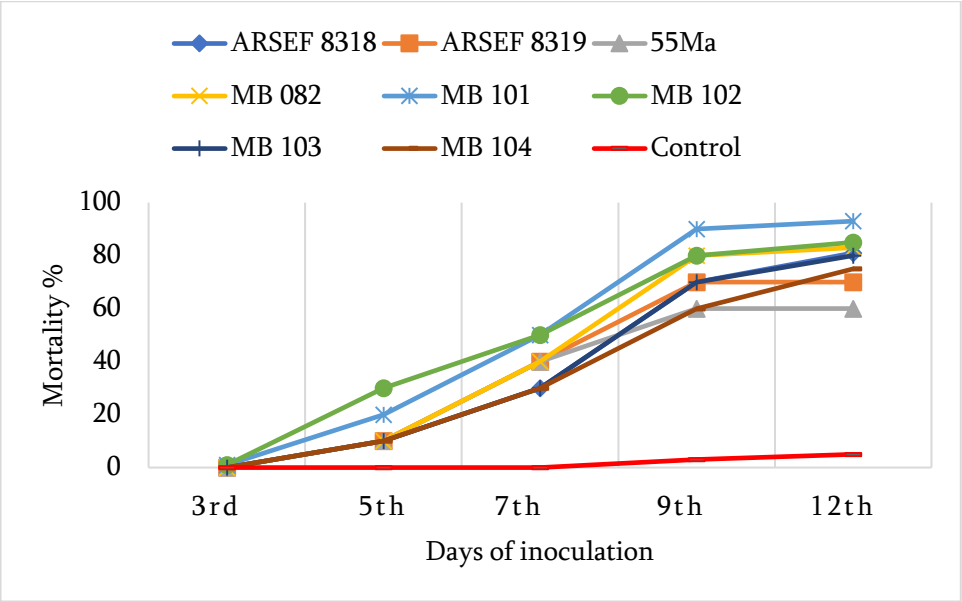


Fig. 39. Cumulative mortality of the local and alien fungal isolates against *H. halys* adults according alternative days, at the concentration 1x10 ^{7}

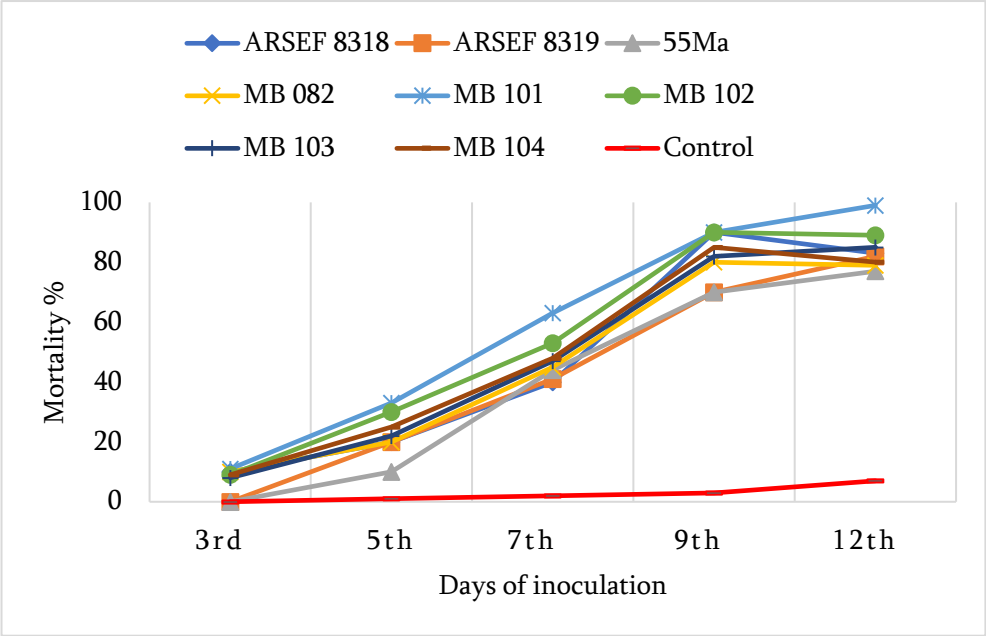


Fig. 40. Cumulative mortality of the local and alien fungal isolates against *H. halys* adults according alternative days, at the concentration 1x10 ^{8}

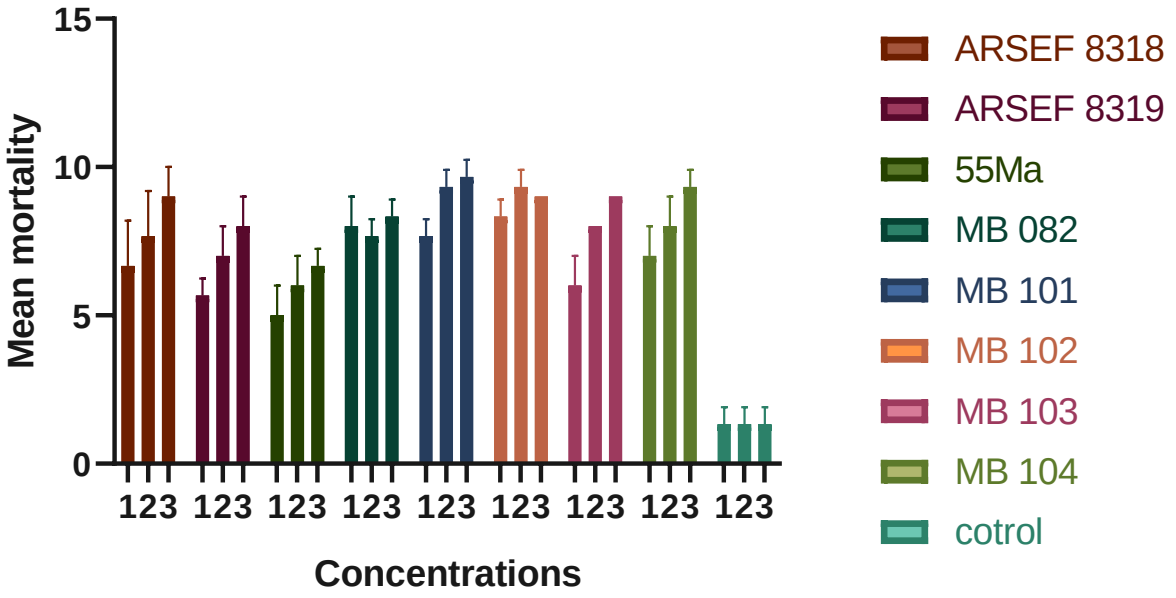


Fig. 41. Mean of the cumulative mortality of the local and alien fungal isolates against *H. halys* adults, at the concentration: **1**- 1x10 ^{6}, **2**- 1x10 ^{7}, **3**-1x10 ^{8}

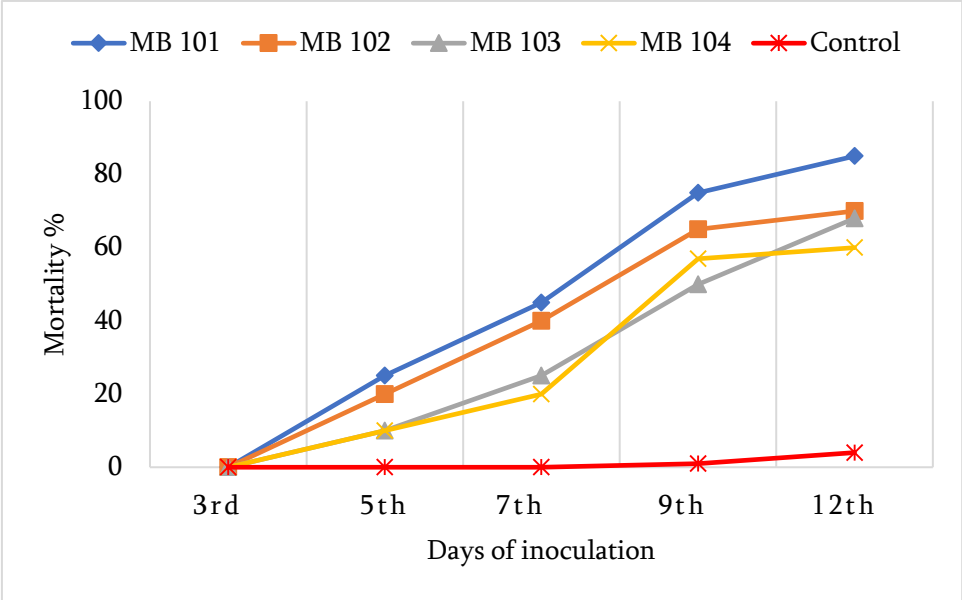


Fig. 42. Cumulative mortality of the *B. bassiana* and *I. fumosorosea* isolates against nymphs of *H. halys* according alternative days, at the concentration 1x10 ^{6}

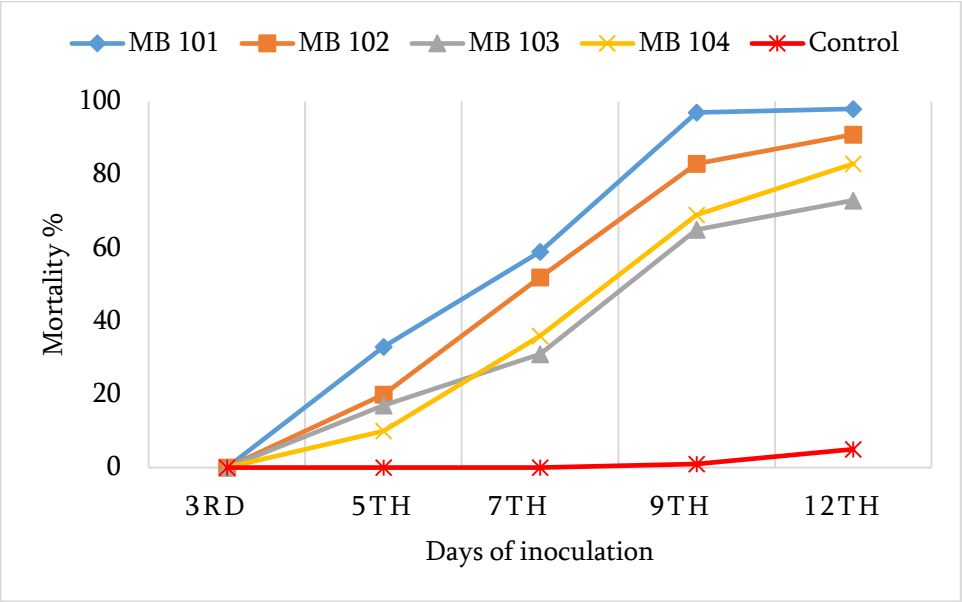


Fig. 43. Cumulative mortality of the *B. bassiana* and *I. fumosorosea* isolates against nymphs of *H. halys* according alternative days, at the concentration 1x10 ^{7}

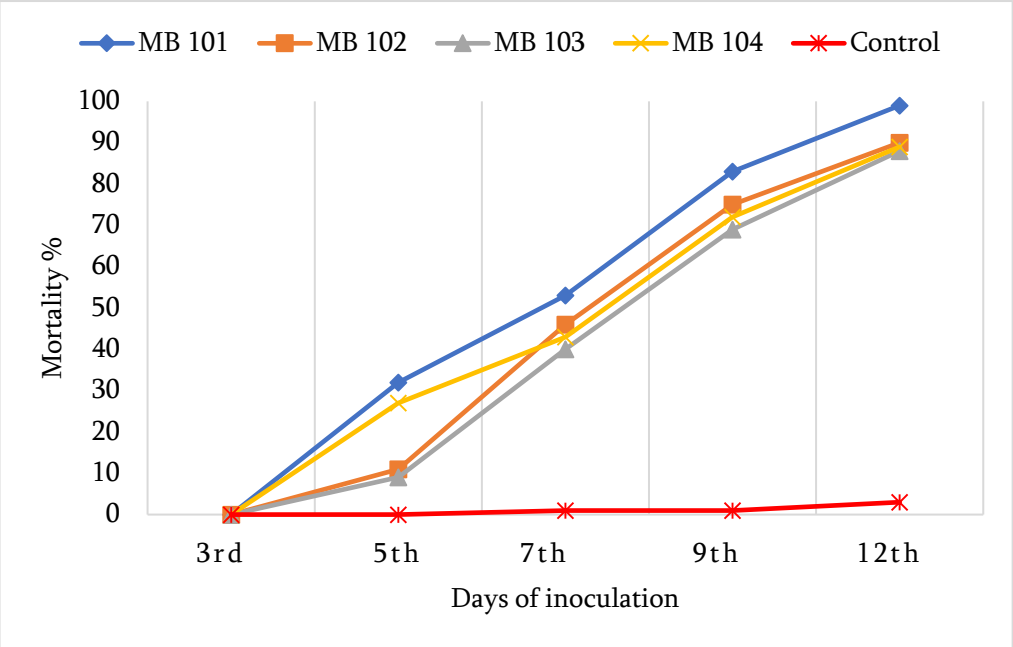


Fig. 44. Cumulative mortality of the *B. bassiana* and *I. fumosorosea* isolates against nymphs of *H. halys* according alternative days, at the concentration 1x10 ^{8}

Table 16. Median lethal concentration LC50 of *H. halys* adults treated by local and alien fungal isolates (ANOVA, P ≤ 0.05)

Isolates	LC50	95%Fiducial CI		Slope	Intercept	χ^2 (df = 2)
		Lower	Upper			

ARSEF 8318	46700,321	1944,39341	1121645,415	0,378	3,233	0,991
ARSEF 8319	146180,88	3488,51310	6125488,930	0,294	3,481	0,998
55MA	1026264,3	18442,8	57107147,9	0,262	3,424	1,000
MB 082	331,0	1,2	93409,7	0,219	4,447	0,736
MB 101	12215,8	502,6	296932,7	0,440	3,202	0,961
MB 102	71,35	0,18	28258,04	0,219	4,593	0,764
MB 103	288125,99	28907,40	2871810,91	0,514	2,194	0,993
MB 104	46700,32	1944,39	1121645,41	0,378	3,233	0,991

Table 17. Median lethal concentration LC50 of *H. halys* nymphs treated by local fungal isolates (ANOVA, $P \leq 0.05$)

Isolates	LC50	95%Fiducial CI		Slope	Intercept	χ^2 (df = 2)
		Lower	Upper			
MB 101	1221579,0	61207,70	24380190,30	0,431	2,3222	0,683
MB 102	1221579,0	61207,70	24380190,30	0,440	2,322	1,000
MB 103	9444376,0	1009173,01	88385476,36	0,512	1,431	0,643
MB 104	25026141	1950753,74	321059364,7	0,421	1,884	0,702

Time to mortality was measured through routine post-treatment observations. Higher rates plateaued faster than lower rates (Figures 38 - 44). The earliest mortality recorded was with the highest concentration of all strains on two and three days after treatment. Mortality analyzed by the test of equality with the strata statement in ´log (survival probability) PROC LIFETEST indicated significant differences between concentrations for all isolates (Figures 45-56).

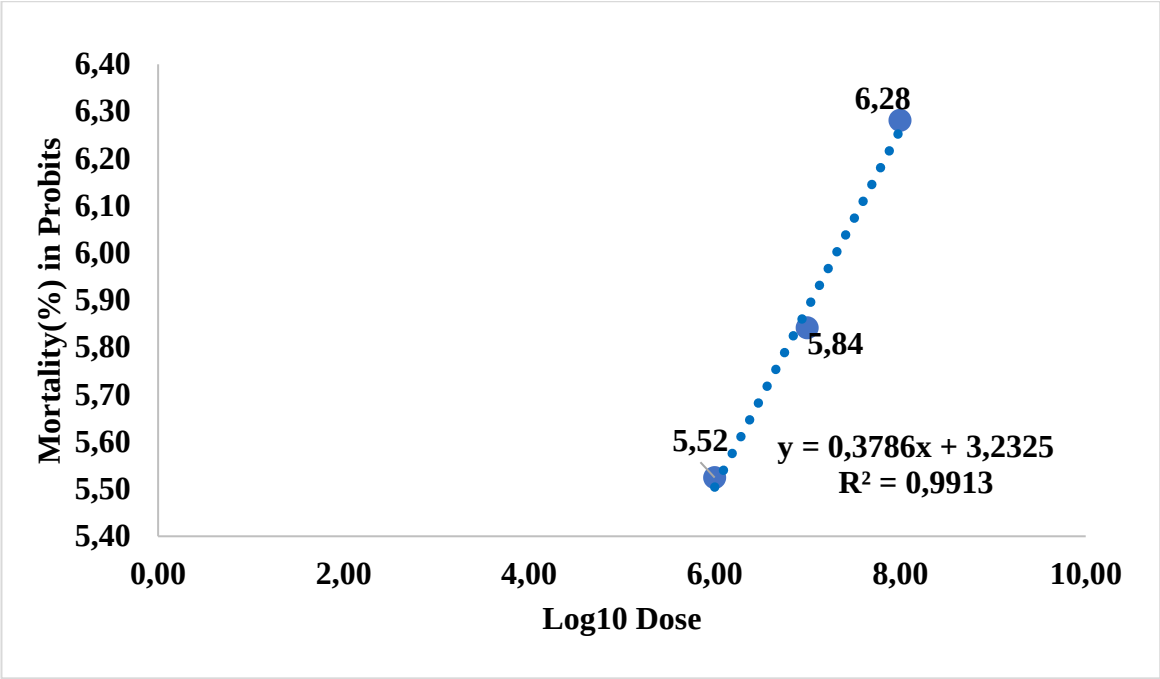


Fig.45. Log concentration probit mortality response of *H. halys* to *Beauveria bassiana* (strain ARSEF 8318)

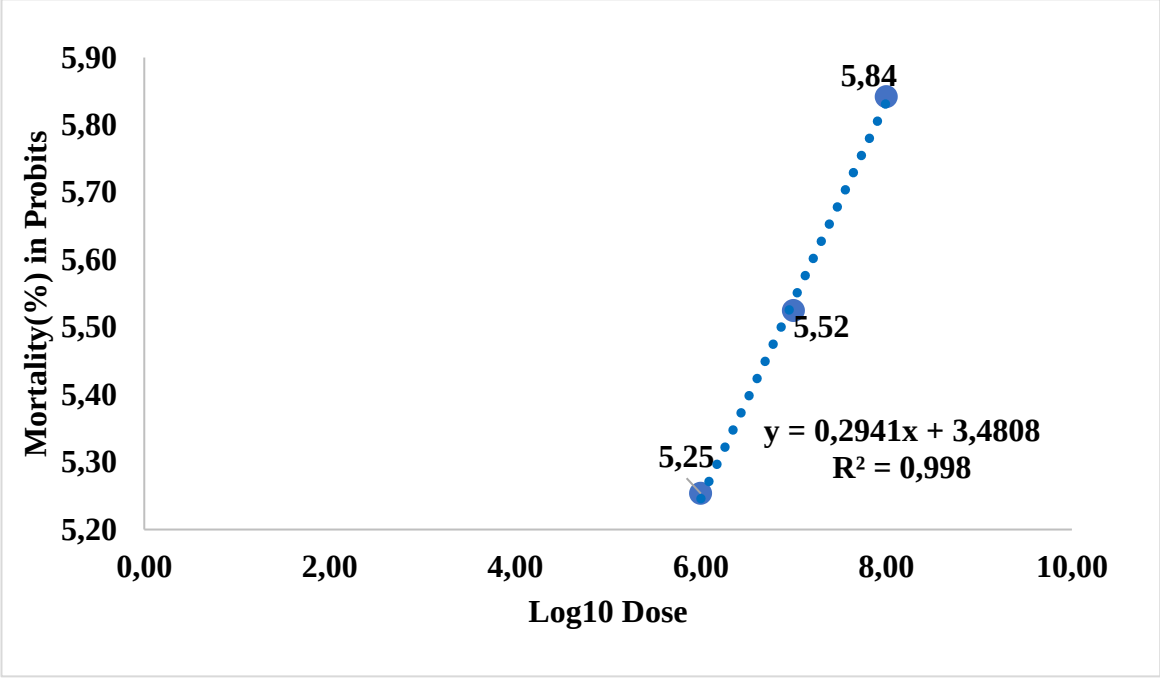


Fig.46. Log concentration probit mortality response of *H. halys* to *Metarhizium anizopliae* (strain ARSEF 8319)

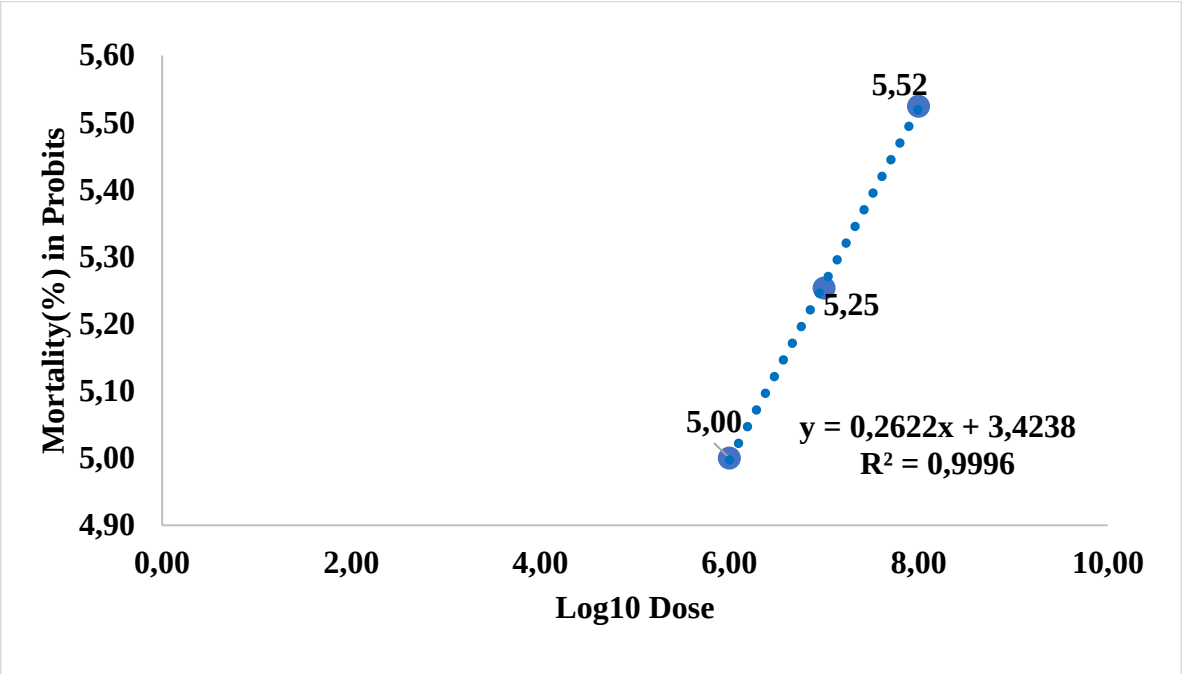


Fig.47. Log concentration probit mortality response of *H. halys* to *Metarhizium anisopliae* (strain 55MA)

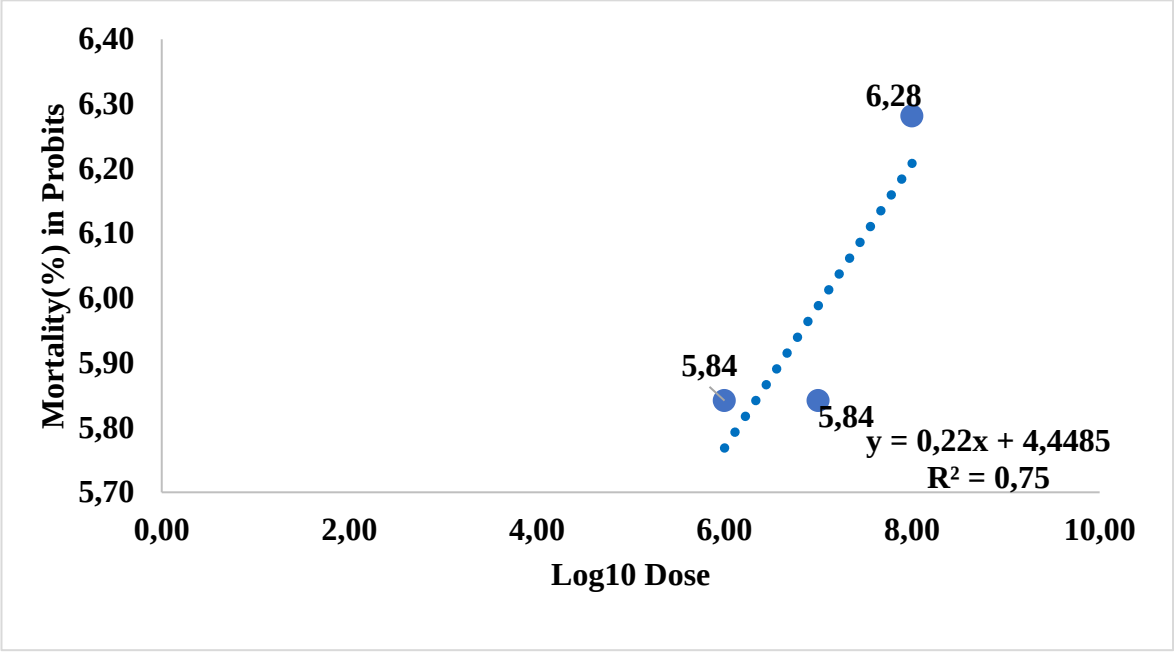


Fig. 48. Log concentration probit mortality response of *H. halys* to *Beauveria bassiana* (strain MB 082)

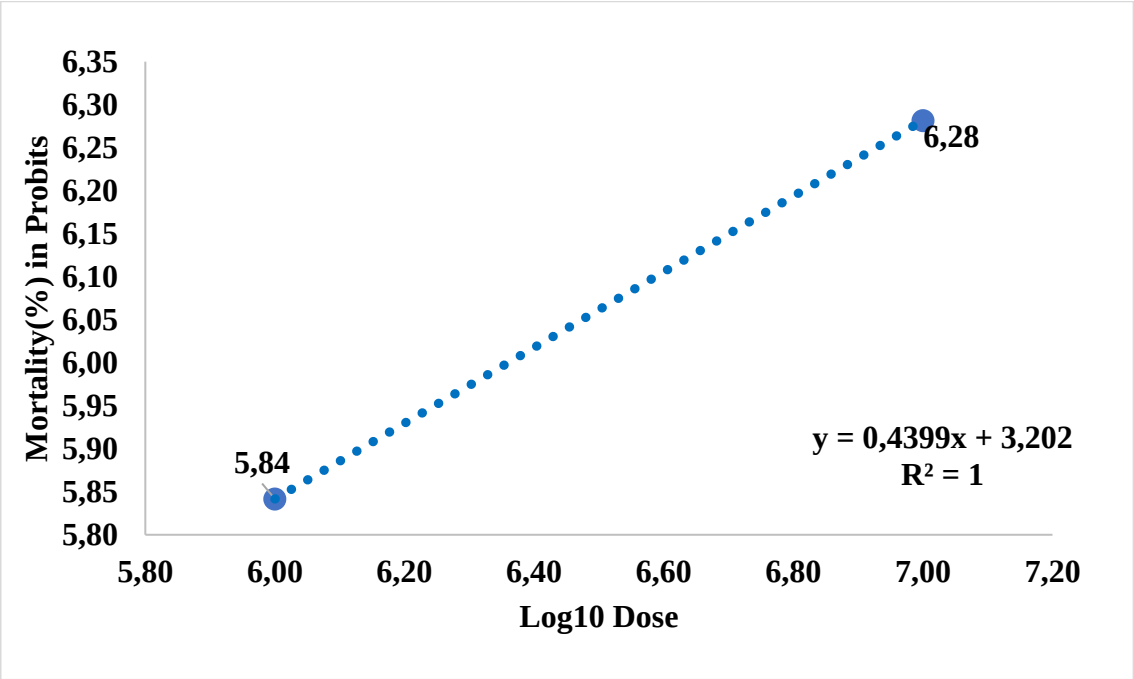


Fig.49. Log concentration probit mortality response of *H. halys* to *Beauveria bassiana* (strain MB 101)

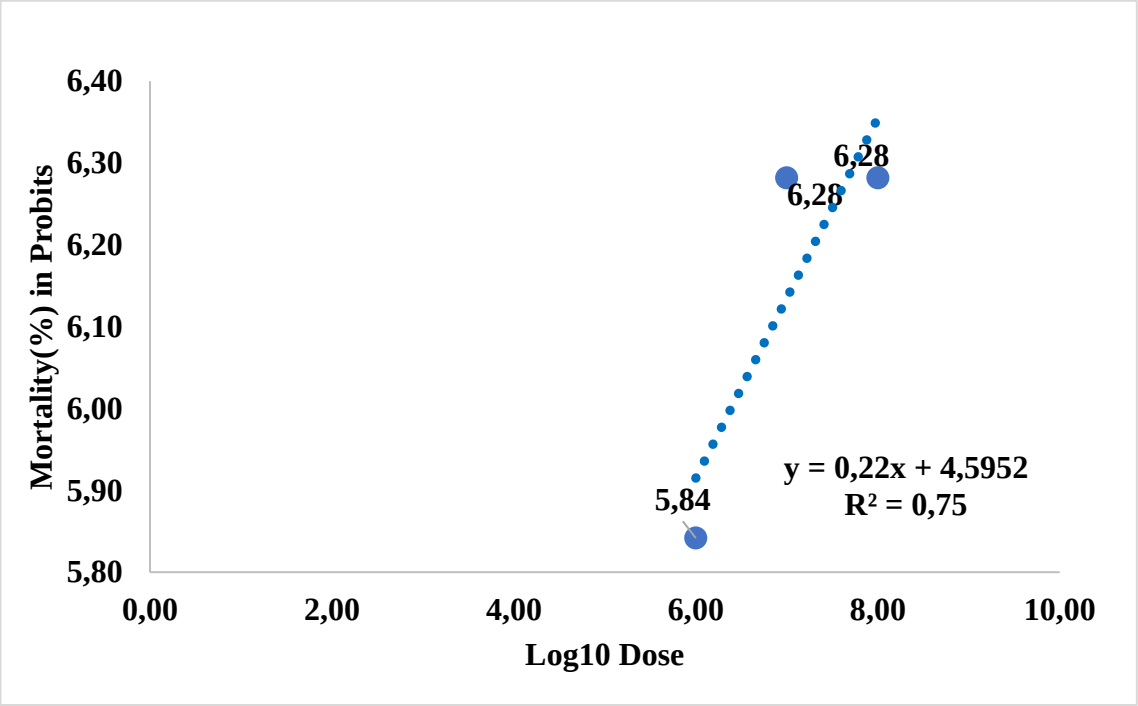


Fig. 50. Log concentration probit mortality response of *H. halys* to *Beauveria bassiana* (strain MB 102)

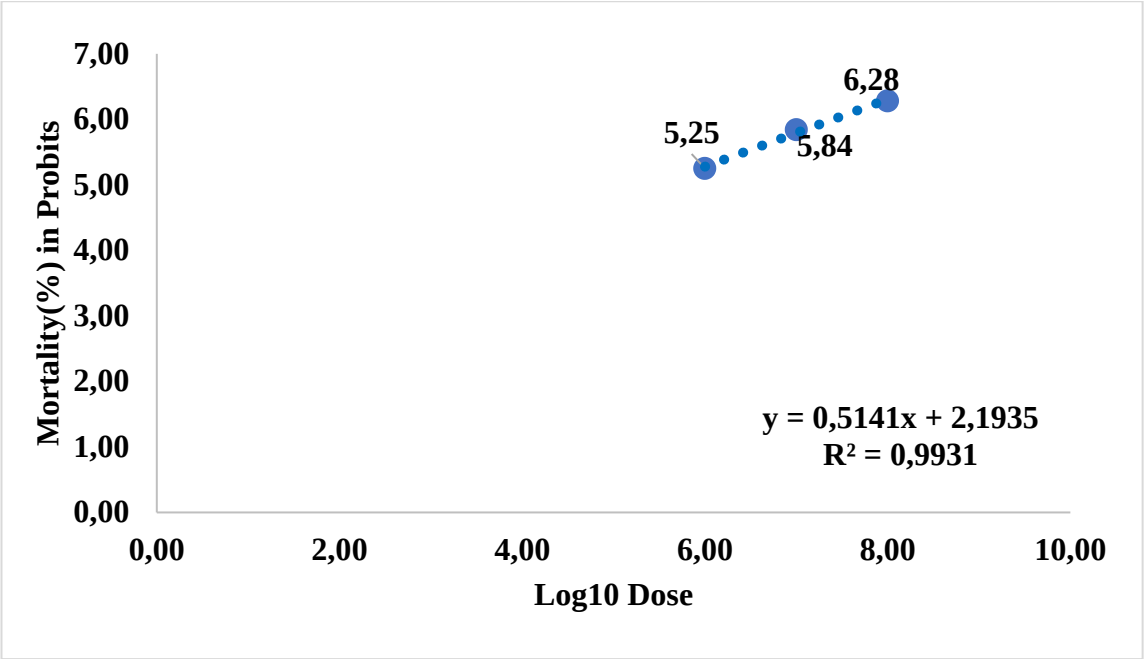


Fig. 51. Log concentration probit mortality response of *H. halys* to *Isaria fumosorosea* (strain MB 103)

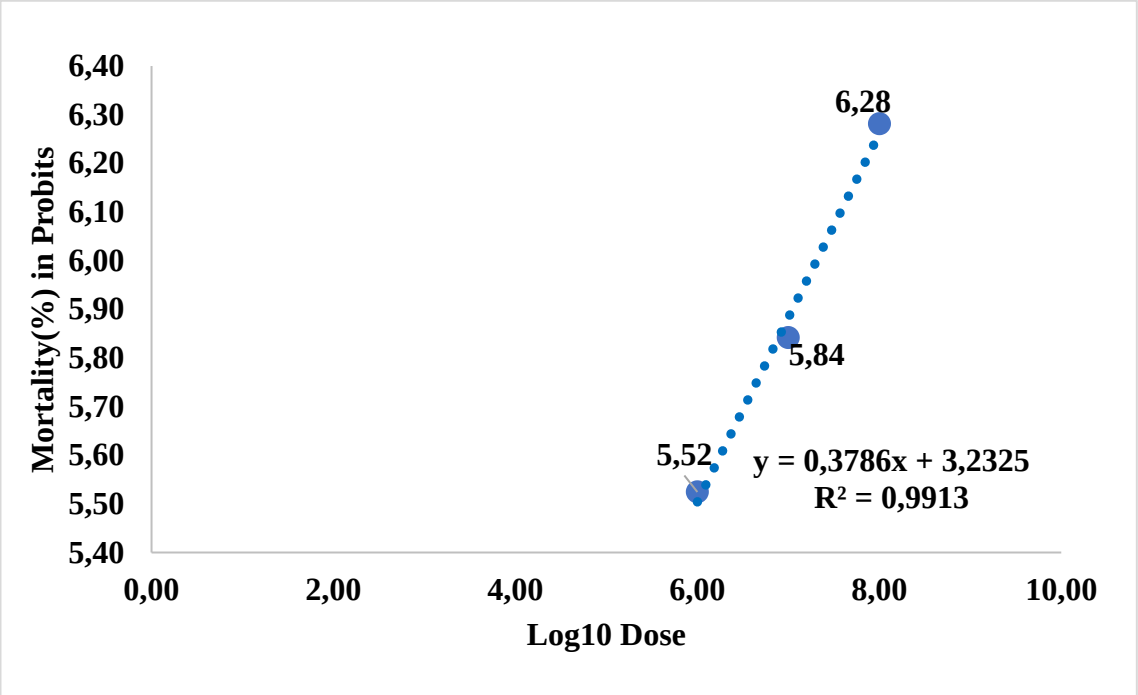


Fig. 52. Log concentration probit mortality response of *H. halys* to *Beauveria bassiana* (strain MB 104)

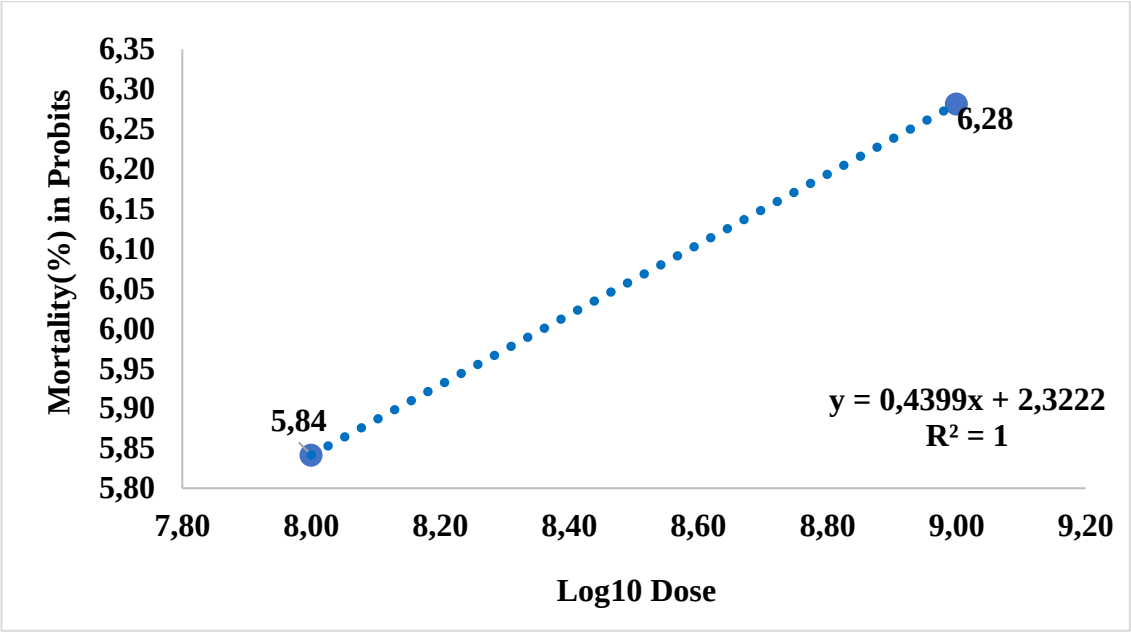


Fig. 53. Log concentration probit mortality response of *H. halys* nymph to *Beauveria bassiana* (strain MB 101)

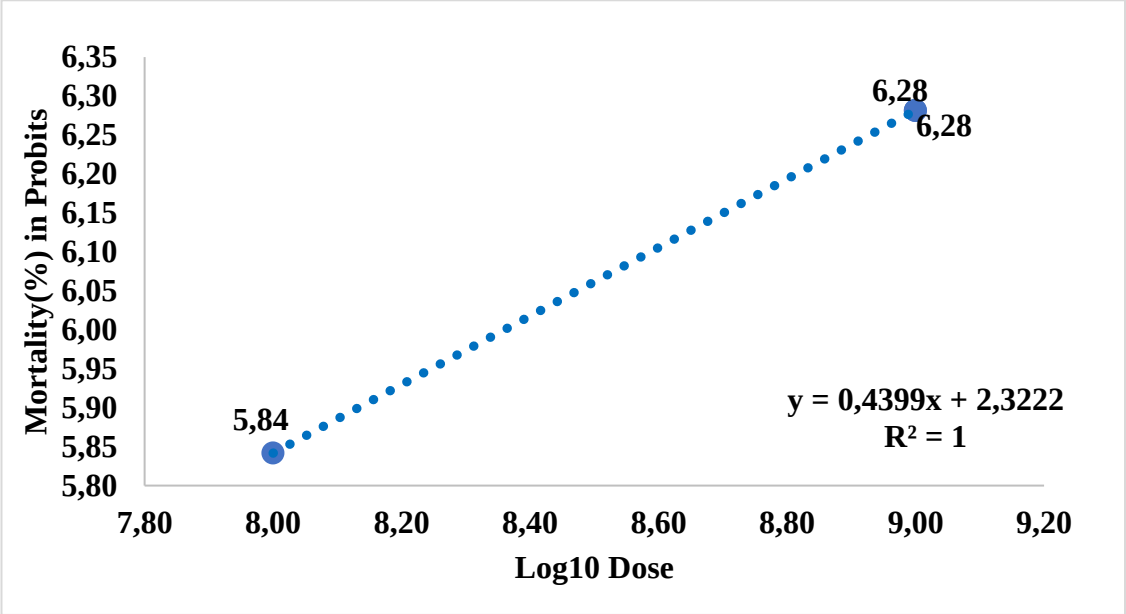


Fig. 54. Log concentration probit mortality response of *H. halys* nymph to *Beauveria bassiana* (strain MB 102)

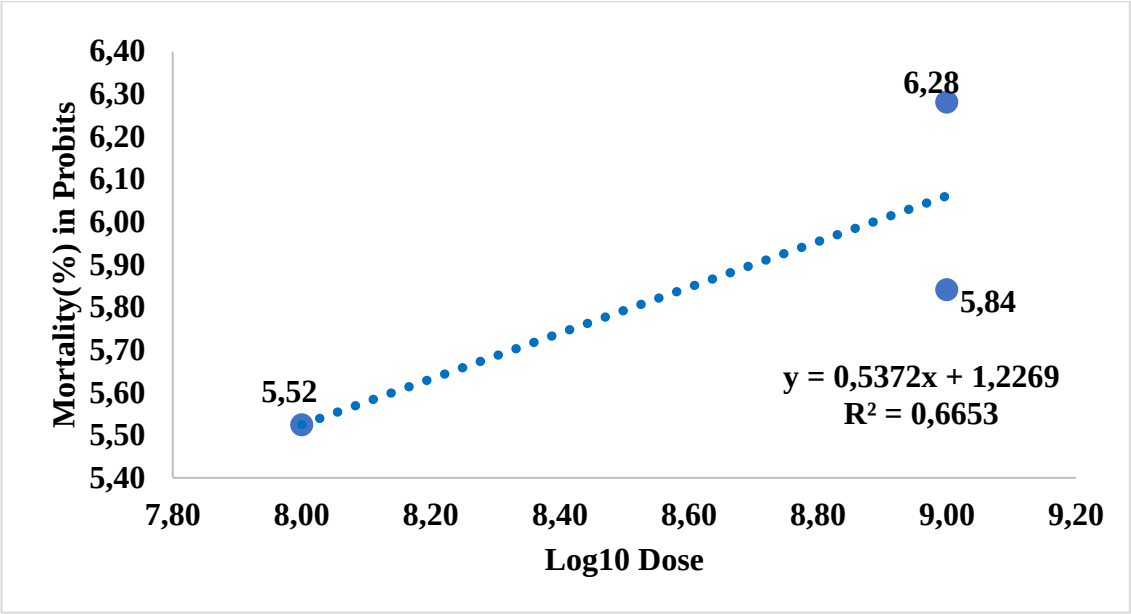


Fig. 55. Log concentration probit mortality response of *H. halys* nymph to *Isaria fumosorosea* (strain MB 103)

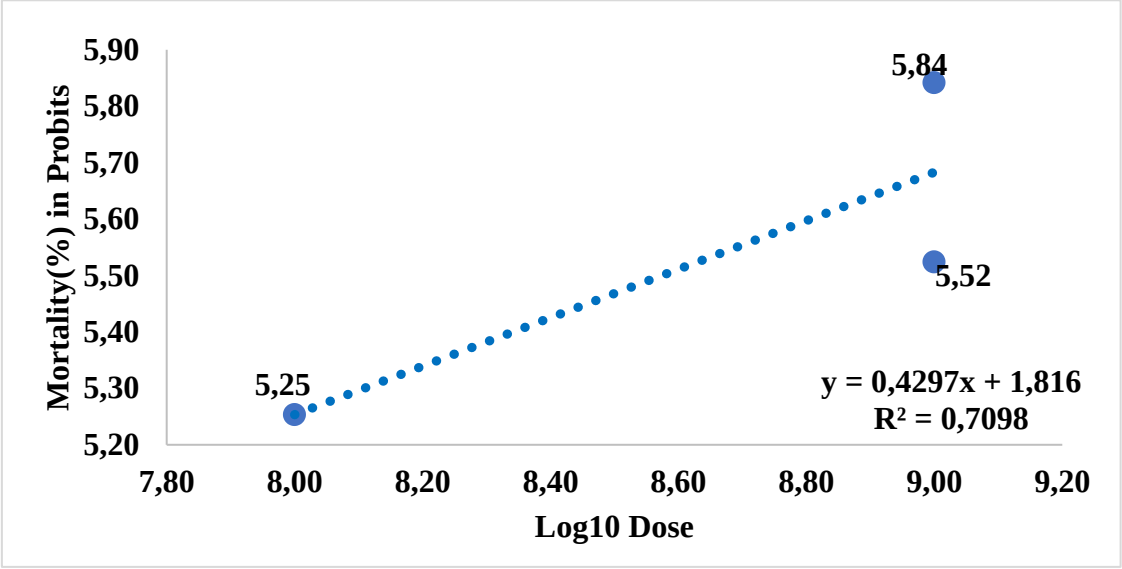


Fig. 56. Log concentration probit mortality response of *H. halys* nymph to Beauveria *bassiana* (strain MB 104)

Table 18. Means and Medians for Survival Time at the concentration 1X10 ^{6} (Adult)

			Mean[a] 95% Confidence Interval				Median 95% Confidence Interval	
Isolates	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
ARSEF 8318	6.108	0.450	5.226	6.990	5.000	0.674	3.680	6.320
ARSEF 8319	6.350	0.447	5.475	7.225	5.000	0.666	3.695	6.305
55MA	6.619	0.465	5.708	7.530	7.000	0.631	5.764	8.236
MB 082	5.857	0.434	5.006	6.708	5.000	0.586	3.852	6.148
MB 101	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
MB 102	6.205	0.433	5.356	7.055	5.000	0.657	3.712	6.288
MB 103	6.488	0.457	5.592	7.383	7.000	0.613	5.798	8.202
MB 104	6.195	0.414	5.385	7.006	5.000	0.610	3.805	6.195
Overall	6.256	0.156	5.951	6.561	5.000	0.233	4.544	5.456

a. Estimation is limited to the largest survival time if it is censored.

Table 19. Means and Medians for Survival Time at the concentration 1X10 ^{7} (Adult)

			Mean[a] 95% Confidence Interval				Median 95% Confidence Interval	
Isolates	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
ARSEF 8318	6.205	0.433	5.356	7.055	5.000	0.657	3.712	6.288
ARSEF 8319	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
55MA	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
MB 082	5.973	0.420	5.150	6.796	5.000	0.638	3.749	6.251
MB 101	5.971	0.442	5.104	6.839	5.000	0.693	3.641	6.359
MB 102	5.848	0.459	4.949	6.748	5.000	0.631	3.763	6.237

MB 103	6.205	0.433	5.356	7.055	5.000	0.657	3.712	6.288
MB 104	6.366	0.436	5.512	7.220	7.000	0.567	5.888	8.112
Overall	6.113	0.153	5.813	6.414	5.000	0.234	4.542	5.458

a. Estimation is limited to the largest survival time if it is censored.

Table 20. Means and Medians for Survival Time at the concentration 1X10 ^{8} (Adult)

	Mean[a]		95% Confidence Interval		Median		95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
ARSEF 8318	5.794	0.417	4.976	6.612	5.000	0.643	3.739	6.261
ARSEF 8319	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
55MA	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
MB 082	5.971	0.443	5.104	6.839	5.000	0.693	3.641	6.359
MB 101	5.758	0.429	4.917	6.598	5.000	0.631	3.763	6.237
MB 102	5.656	0.430	4.814	6.499	5.000	0.617	3.790	6.210
MB 103	5.971	0.442	5.104	6.839	5.000	0.693	3.641	6.359
MB 104	5.971	0.442	5.104	6.839	5.000	0.693	3.641	6.359
Overall	5.932	0.153	5.633	6.231	5.000	0.225	4.559	5.441

a. Estimation is limited to the largest survival time if it is censored.

Table 21. Means and Medians for Survival Time 1X10 ^{6} nymph

	Mean[a]		95% Confidence Interval		Median		95% Confidence Interval	
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
MB 101	6.108	0.450	5.226	6.990	5.000	0.674	3.680	6.320
MB 102	6.263	0.465	5.353	7.174	5.000	0.684	3.659	6.341

MB 103	6.488	0.457	5.592	7.383	7.000	0.613	5.798	8.202
MB 104	6.548	0.450	5.666	7.429	7.000	0.631	5.764	8.236
Overall	6.361	0.226	5.918	6.804	5.000	0.340	4.334	5.666

a. Estimation is limited to the largest survival time if it is censored.

Table 22. Means and Medians for Survival Time 1X10 ^{7} nymph

	Mean[a] 95% Confidence Interval				Median 95% Confidence Interval			
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
MB 101	5.636	0.417	4.819	6.454	5.000	0.561	3.900	6.100
MB 102	5.857	0.434	5.006	6.708	5.000	0.586	3.852	6.148
MB 103	6.350	0.447	5.475	7.225	5.000	0.666	3.695	6.305
MB 104	6.350	0.447	5.475	7.225	5.000	0.666	3.695	6.305
Overall	6.074	0.219	5.645	6.504	5.000	0.332	4.349	5.651

a. Estimation is limited to the largest survival time if it is censored.

Table 23. Means and Medians for Survival Time 1X10 ^{8} nymph

	Mean[a] 95% Confidence Interval				Median 95% Confidence Interval			
	Estimate	Std. Error	Lower Bound	Upper Bound	Estimate	Std. Error	Lower Bound	Upper Bound
MB 101	5.824	0.446	4.950	6.697	5.000	0.574	3.875	6.125
MB 102	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
MB 103	6.132	0.438	5.272	6.991	5.000	0.648	3.730	6.270
MB 104	6.108	0.450	5.226	6.990	5.000	0.674	3.680	6.320
Overall	6.054	0.220	5.624	6.485	5.000	0.331	4.351	5.649

a. Estimation is limited to the largest survival time if it is censored.

4.16.5 Biological efficiency of isolated EPF in field

After ten days of treatment, the mortality ranged from 41.8%, to 79.0%. The maximum mortality (79.0 %.) was recorded in *B. bassiana* MB 101 and was significantly better than all other treatments. This was followed by ARSEF 8319 - which recorded 62.8%, 55Ma- 53.8%, MB102 -52.6%, ARSEF 8318 - 50.9%, The minimum mortality (41.8%,) was recorded in *I.fumosorosea* - MB103. All treatments were significantly different than the control 9.2%, Control (Dimethioate) -100 % (Fig. 57).

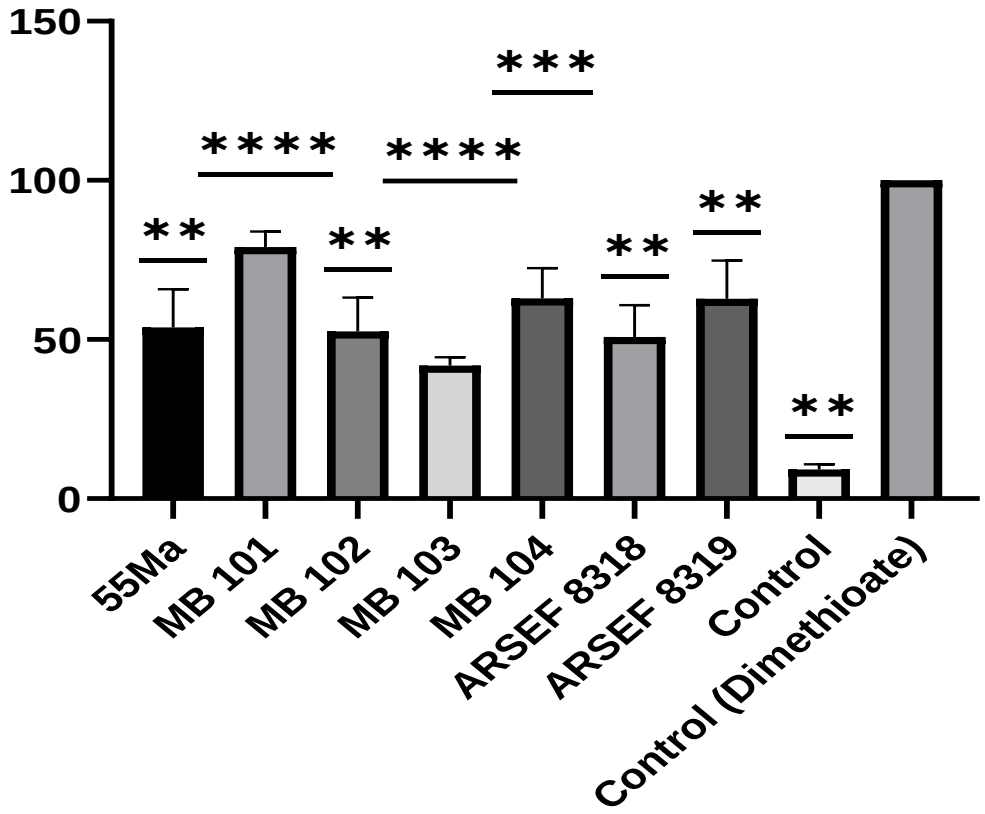


Fig. 57. Average mortality of entomopathogenic fungi isolated from *H. halys* - *B. bassiana* - MB 101, MB 102, MB 104 and *I. fumosorosea* - MB 103, Metarhizium sp.– 55MA from local collection, and alien strains of *B. bassiana* – ARSEF 8318 *and Metarhizium sp.* ARSEF 8319

Table 24. Descriptive statistics of field trials

	Minimum	Maximum	Range	Mean	Std. Deviation	Std. Error	Lower 95% CI	Upper 95% CI	Coefficient of variation
55Ma	40,83	65,00	24,17	53,80	12,02	6,010	34,67	72,93	22,34%
MB 101	74,16	85,00	10,84	79,01	4,939	2,470	71,15	86,87	6252%
MB 102	42,50	66,00	23,50	52,59	10,62	5,310	35,69	69,49	20,19%
MB 103	39,16	45,00	5,840	41,79	2,701	1,351	37,49	46,09	6464%
MB 104	52,66	72,00	19,34	62,92	9,537	4,769	47,74	78,09	15,16%
ARSEF 8318	40,25	60,50	20,25	50,86	9,911	4,955	35,09	66,63	19,49%
ARSEF 8319	50,00	75,00	25,00	62,75	12,12	6,060	43,46	82,04	19,32%
Control	7,500	11,00	3,500	9,200	1,590	0,7948	6,671	11,73	17,28%
Control (Dimethioate)	100,0	100,0	0,000	100,0	0,000	0,000	100,0	100,0	0,000%

The infected bugs showed visible mycosis characterized by the development of fungal mycelia on the insect body (Fig.58).

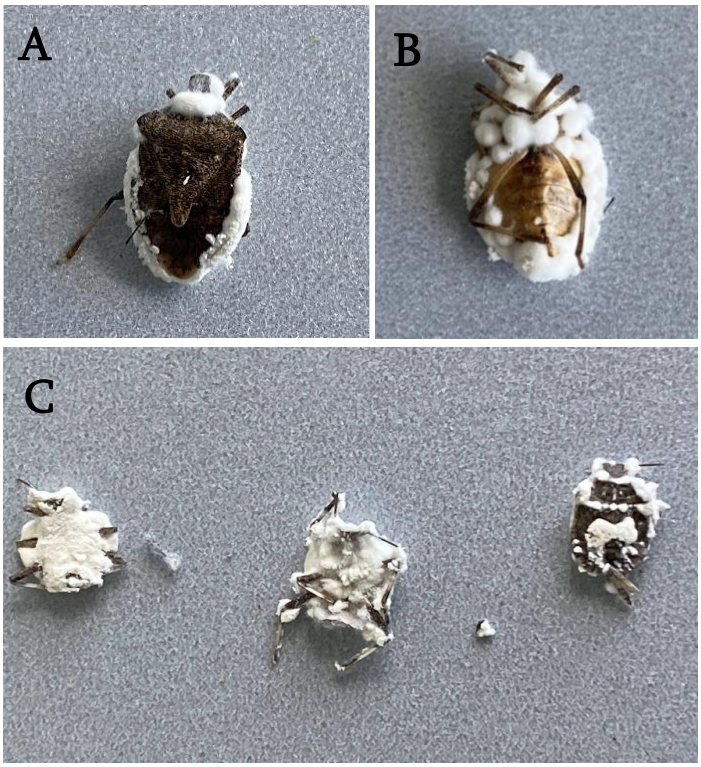


Fig. 58. Fungal micelia developed on the *H. halys* A,B- adults, C- nymphs

4.17 Discussion

The annual number of *H. halys* generations vary according to the geographical climate conditions. In the native regions of *H. halys* such as China, Korea, Japan different number of generations develop. One to six generation occurrences is predicted in distinct locations in China (Hoffman, 1931). One or two generations have been reported in Japan as well (Lee et al., 2013). Watanabe (1980) assumed that *H. halys* is univoltine in western and bivoltine in eastern Japan. In the mid-Atlantic region of the USA, it has one or two generations per year (Nielsen et al., 2008). In Switzerland, *H. halys* is univoltine, but if insect continues to spread into the Mediterranean area two generations per year could be expected (Haye et al., 2014). Abiotic conditions during the growing season affect the number of generations produced per year (Kistner, 2017). Insect phenology is primarily driven by temperature and photoperiod (Bale et al., 2002; Tobin et al., 2008), which can vary with latitude and other geographical parameters.

Number of generations of *H. halys* during one vegetation period in the subtropical zone of Western Georgia (WG) is controversial subject. According to Karpun & Perchenko (Karpun, 2016), on the east coast of the Black Sea basin, which is characterized as subtropical climate: in the Krasnodar, Sochi, Abkhazia, Adjara, *H. halys* develops three generations. In the subtropical zone of Azerbaijan - Lenkoran, Absheron and Zakartala - Z. Mamedov (Mamedov, 2018) reported three annual generations as well. By the dates of M. Murvanidze (Murvanidze et al., 2018) and E. Jakeli (Jakeli and Nikolaishvili, 2019), *H. halys* develops two generations in hazelnut orchards of WG. However, for our investigation three generations of *H. halys* were observed in the subtropical zone of WG (Guria, Samegrelo) in 2018. Additionally, we monitored not only hazelnut orchards, but maize fields, citrus orchards, ornamental trees, other crops and wild plants.

Based on the various research conducted in Georgia it can be concluded that *H. halys* develops two generations, but in the presence of favorable climatic conditions it can complete the development of the third generation.

H. halys feeding has been observed on 38 species of plants, which include 23 families and 31 genera. Three species as a host plant *Agava americana* L., *Sambucus nigra* L., *Laurus*

nobilis L. were identified for the first time in the conditions of WG. During the growing season *H. halys* prefers different types of plants for feeding. Mid April (after overwintering) to end of May *H. halys* were observed in the Citrus orchards (2022-2023 years). From May to the end of June, *H. halys* mainly feeds on hazelnuts. From July to the end of August it prefers vegetables and corn. From August to October, its feeding was described on fruit trees (persimmon, pear, grape, plum) and wild herbaceous plants.

One of the objectives of our research was to study the damage and resistance of hazelnut as an economic important crop for Georgia. The study revealed that hazelnuts are damaged by nymphs and adults of *H. halys*. From the host plants, most egg masses were found on hazelnuts, indicating that hazelnuts are dominant host plant. Mostly egg masses were found on the second or third leaves of fruitful hazelnut branches. This fact should be explained by plant physiology. A fruiting hazelnut branch should contain more nutrients than a branch with male flowers. Therefore, the insect chooses a place rich in nutrients. Feeding damages are well expressed on the fruits, but a lesser extent occurs on the leaves. The degree of fruit damage depends on the age and generation of *H. halys*, although the length of its rostum is always greater than the thickness of the nut shell, which allows the insect to damage the nut fruit at all ages (Kharabadze et al., 2022). *H. halys* saliva contains various enzymes necessary for digestion, but it does not contain lignin-degrading enzymes (Peiffer, 2014, Will, 2012). The insect is unable to damage lignified shells of the almond and pistachios as well (Rijal, 2018, Jesús et al., 2017). Therefore, once the lignification process begins in the nut shell, the nut fruit becomes unacceptable for the *H. halys* feeding. Shell lignification begins at different times in hazelnut varieties and varies according to meteorological conditions (Boudet, 2000; Moura, 2010; Moura-Sobczak, 2011; Lauvergeat, 2001; Hano, 2005; Liu, 2018). The ability to produce lignin is genetically determined and varies by cultivar (Osakabe, 1999). According to our experiments the starting time and duration of the lignification process are very important for the hazelnut resistance to *H. halys*. More damaged hazelnut variety Tita consists of less lignin than the less damaged Berdznula.

Seventy-two species of Pentatomidae were observed in Georgia (Zaitseva, 1998). After invasion and population growth of *H. halys*, they became food source for native predator species. The following predators from *H. halys* populations were identified - *Rhynocoris*

iracundus, Hierodula transcaucasica, Iris polistictica. Further studies continued with *H. transcaucasica* established that it can be used as one of the biological control tools within IPM. *H. transcaucasica* is considered to be the most common mantis spread to Easternmost Europe (Harz & Kaltenbach, 1976). Nowadays, the size and area of *H. transcaucasica* population is also increasing in the Black Sea region due to climate change and global warming (Pushkar and Kavurka, 2016; Harz and Kaltenback, 1976).

Currently the role of *H. transcaucasica* in Georgia and European ecosystems is unstudied. But it is known that *H. transcaucasica* is a voracious predator, can be considered to eat more than *Mantis religiosa* and is capable of attacking a large number of invertebrates and small vertebrates (Battiston et al., 2018, Prete et al., 1999). This ability allows it to adapt and be competitive in different environmental conditions (Battiston et al., 2018). Because of their beneficial characteristics, they are used as natural pest control agents in sustainable farming systems. In some countries mantis ootheca is produced to be sold as a biological pesticide (Lelito, 2015; Mahr et al., 2008).

During 2018-2020 it was observed that mantis was following *H. halys* gathering and migrating population in the outdoor and indoor. Laboratory studies have shown that *H. transcaucasica* is predating on *H. halys* in all stages of post-embryonic development.

Matsura et al. (1975; 1984) investigated the relationships among prey consumption, instar duration, body size and food assimilation efficiency in each stage of the congeneric praying mantis (Iwasaki 1991). The study of predatory behavior based on the expression and model of adult mantises is reflected in the experiments of various scientists (Rilling et al., 1959; Holling, 1964; Maldonado et al., 1970; Maldonado and Rodriguez, 1971; Copeland and Carlson, 1979; Iwasaki, 1991).

According to Battiston and Massa (2008) morphological description the length of *H. transcaucasica* ranges from 45-63.3 mm, while according to our data, lengths are 65-72 mm. No other significant differences were found between the literature data and in the results obtained from our experiments.

Predation of mantises was studied by Iwasaki (1991), using nymphs of all ages that underwent starvation periods. With this model, he established relationships between the

maximum volume of prey and body length and as the mantis became larger, it attacked the larger prey model.

In our laboratory predation experiments were established in the adults and nymphs of *H. transcaucasica* after they underwent starvation periods. 3rd instar nymph of *H. transcaucasica* preyed on 1st instar *H. halys* nymphs, while adult *H. transcaucasica* attacked the adult of *H. halys*. The maximum number of *H. halys* (7 insect) was devastated by an adult *H. transcaucasica* on the first day, and in this case the same number of nymphs (7 insects) within the first hours of the experiment - 9:30 am - 12:30 pm were observed.

During the experiment, when adults of *H. halys* and *H. transcaucasica* were inserted into the insect rearing cage, it was observed that *H. transcaucasica* was mostly standing at one place, while *H. halys* moved towards it. This may be caused by vibrational signals emitted by the predator. Vibration signals are known to be used by insects for communication and predation (Mazzoni et al., 2017; Mazzoni et al., 2009; Mazzoni et al., 2014; Pollac & Ollack, 2000).

As a summary, *H. transcaucasica* is one of the most significant natural enemies of *H. halys*. But more research is needed in this area. The study of mantis behaviors in their relationship to target insects is more promising and could be used in biological control.

For the first time three strains of entomopathogenic fungi (EPF) MB 101, MB 102, MB 104 - *Beauveria bassiana* and one strain MB 103 -*Isaria fumosorosea* were isolated from *H. halys* WG populations and their species were identified by morphological study. The parameters, such as conidial germination and the production of hydrolytic enzymes are associated with the virulence of EPF (Petrisor and Stoian, 2017; Faria et al., 2015). The virulence of entomopathogenic fungi is defined by extracellular cuticle-degrading enzymes including proteases, chitinases, and lipases (Cheong et al, 2016). Enzyme analysis confirmed that the isolated strains (*Beauveria bassiana* - MB101; MB102; MB104; *Isaria fumosorosea* - MB 103) have high chitinase, protease and lipase activities.

To establish efficiencies of isolated EPFs, assays were performed in the laboratory against *H. halys* adults and nymphs. The same local isolates and alien strains (ARSEF 8318 - *Beauveria bassiana*, ARSEF 8319 - *Metarhizium anisopliae* s.l.) were applied in field experiments.

Mortality of the *H. halys* nymphs using the strains MB 101, MB 102, MB 103, MB 104 varied 85-99%, 70-91%, 68-88%, 60-89% respectively. Comparing to the mortality of the adult MB 101- 80-99%, MB 102 - 75-89%, MB 103 - 60-85%, MB 104 - 70-80% using the same strains and concentrations, demonstrates that this strains are more pathogenic against nymph stages of *H. halys*. According to Parker et al. (2015) nymphs of *H. halys* were more affected by fungi infection than the adults. In every experiment highest mortality were observed by strain MB 101 – *Beauveria bassiana.* V. V. Gouli (2012) have reported that the *Beauveria bassiana* (67-100%) is more effective entomopathogenic fungi than *Metarhizium anisopliae* (40-88%) against *H. halys*.

Experiments showed that mortality rate was directly connected to suspension concentration ($1x10^6$, $1x10^7$, and $1x10^8$). Means and medians for survival time were decreasing while spore concentrations were increased. Maximum mortality was recorded at higher concentrations ($1x10^8$) of spores on all 12 days of observations. Analogical results are reported in different publications (Butt and Goettel, 2000; Akmal et al., 2013).

The strain MB 101 with the highest enzymatic activity was characterized by high biological efficiency. A positive correlation between production of extracellular enzymes and mortality rate was reported in other research (Dhawan and Joshi 2017).

We also studied the effectiveness of the first Georgian mycopesticide Bover-Ge against adults and overwintering *H. halys*, where mortality varied from 65 to 90.5%. About the GHA strain (*B. bassiana*) the active ingredient in BotaniGard® tested against *H. halys* was reported in previous studies. Effectiveness of this mycopesticide achieved 85-100% (Gouli et al., 2012; Parker et al., 2015).

Entomopathogenic nematodes are promising biocontrol agents showing high potential for the control of many pest insects (Lacey & Kaya, 2007; Poinar, 1990; Půža, 2015). First report of the local entmopathogenic Steinernematidae nematodes: *S. tbilisiensis*, *S. thesami*, *S. carpocapsae* and Heterorhabditidae: *H. bacteriophora*, against *H. halys* were done in 2017 (Gorgodze et al., 2017). Each strain of EPNs were tested separately as well as in combination with local EPF - *Isaria fumosorosea.* Mortality caused by nematodes varied in nymphs 58.5 - 83.3% and in adults 39.2-73.7%. Effectiveness of EPNs in combination with *I. fumosorosea* varied 78-96% in nymphs and 63-91% in adults of *H. halys*.

According to Mikaia (2018) the virulence of *Steinernema carpocapsae* against *H. halys* is higher than that of *Heterorhabditis bacteriophora* which depends on the time, type and concentration of the nematodes. On the 7th day after treatment maximum mortality achieved 68% and 48% respectively.

In our experiments the potential of native (*Heterorhabditis bacteriophora* (GEO), *Steinernema borjomiense*) and non-native Italian (*Heterorhabditis bacteriophora* (IT), *Steinernema apuliae.*) EPN strains against *H. halys* under laboratory conditions were investigated. Cumulated mortality of *H. halys* adults reached 95.5% for *H. bacteriophora* (IT), 60% for *S. apuliae*, 53.3% for *H. bacteriophora* (GEO) and 40% for S*. borjomiense*.

After invading and successfully establishing its populations in WG, *H. halys* adapts to different climate conditions and continues invasion in new regions of Eastern Georgia (EG). Western and Eastern Georgia differ in terms of climatic conditions and plant cover in forests and agricultural fields. The insect's strong adaptability and the accelerating climate change pose threat of its outbreak in East Georgia as well. Further research on bioecology (host plants, numer of generations and etc.) of *H. halys* will be necessary in the conditions of Eastern Georgia.

5 Conclusion and Recommendations

Conclusions:

- Two or rarely three generations of the *H. halys* are recorded in the conditions of Western Georgia.
- For the first time established that overwintered adults and I-II generation insects prefer hazelnuts for ovipositon. The number of successfully hatched eggs sampled from different plants varied from 75 to 79% by years.
- In *H. halys* overwintering populations the number of females always exceeded males by 6-60% in 2018-2021 years.
- For the first time *H. halys* feeding was observed on 38 species of plants in the conditions of Western Georgia, of which three species *Agava americana, Sambucus nigra, Laurus nobilis* were not included in the list of *H. halys* feeding plants of CABI/EPPO.

- Resistance of the nut fruit depends on the time of initiation of lignification. The lignified shell is a barrier for the insect, which cannot damage it. In the studied cultivars, the insect preferred the cultivar with less lignin content.
- For the first time the following local natural enemies have been identified from *H. halys* WG populations: predators - *Rhynocoris iracundus, Hierodula transcaucasica, Iris polistictica*, Dermeste sp., and from entomopathogenic microorganisms - fungi *Beauveria bassiana, Isaria fumosorosea*.
- An adult *Hierodula transcaucasica* kills an average of three *H. halys* per day, while its nymph kills three *H. halys* nymph per hour in the laboratory.
- On the basis of morphological analyses, the species affiliation of entomopathogenic fungi isolated from *H. halys* populations - *Beauveria bassiana* and *Isaria fumosorosea* - was established.
- The enzymatic (chitinase, lipase and protease) activities of the isolated strains were studied and determined that strain MB 101 is characterized by the highest activity.
- The efficiency of the isolated strains MB 101, MB 102, MB 103, MB 104 against *H. halys* nymphs and adults ranges from 60 to 90%.
- Entomopathogenic fungal isolates of *Beauveria bassiana* - MB 101, MB 102, MB 104 were tested against *H. halys* nymphs and adults at the three concentrations - $1x10^6$, $1x10^7$, $1x10^8$, where mortality was 80-99%, 85-90%, 70-90% respectively, and against imago - 80-99%, 75-89%, 60-80% respectively.
- Biological efficiency of *Isaria fumosorosea* - MB 103 against *H. halys* nymphs varies from 68-88%, and against imago it was 70-90% respectively.
- The biological efficiency of Bover-Ge tested against *H. halys* imago is 72.0-90.5%, and it was 65% in the overwintering phase.
- The biological efficiency of entomopathogenic nematodes *Heterorhabditis bacteriophora* (GEO), *Steinernema borjomiense*, *Heterorhabditis bacteriophora* (IT) and *Steinernema apuliae* against *H. halys* adults reached 53.3%, 40%, 95.3%, 93.3%, respectively.

Recommendations

The presented paper is important from the theoretical and practical point of view, in the field of plant protection, environmental protection, and also in the socio-economic field.

The obtained material and conducted studies allow us to recommend the identified natural enemies as biological agents for the control of *H. halys*. Also recommended are those varieties of nuts that start to accumulate lignin in the shell early and prevent damage to the fruit.

For the first time in the world, we have isolated entomopathogenic fungi from *H. halys* populations. The obtained results ensure the regulation of the number of the pest *H. halys* using environmentally friendly and safe methods.

Today, the agricultural sector in Georgia is an important and developing area for the country's economy. The establishment of farms and greenhouse systems in the future requires integrated protection of agricultural crops from pests and diseases. In case of mass reproduction, the economic losses caused by pests are great. Therefore, the production and timely use of biopreparations and other ecologically safe means created with local resources will significantly reduce costs, increase their effectiveness, and will also be easily available to farmers.

Prospects for further development and application of the thesis:

Clarification of the bioecology of the *H. halys* in the conditions of Georgia, research and study of its local natural enemies will help to strengthen the biological control of this pest, reduce the intensive use of chemical means, obtain environmentally friendly, healthy and competitive products and maintain the ecological balance of the environment. Biopreparations, which will be made on ecologically safe local strains, will be more effective and adapted to environmental variability.

From a commercial point of view: isolated and studied entomopathogenic fungi may be offered to both local and regional biopesticide producers.

The research results have been published in high-ranking international scientific journals:

1. Kharabadze, N., Chkhaidze, N., Abramishvili, T. et al. (2022). First report of Hierodula transcaucasica (Brunner von Wattenwyl, 1878) predation on the Halyomorpha halys (Stål, 1855) in Georgia. Int J Trop Insect Sci 42, 3283–3292 https://doi.org/10.1007/s42690-022-00826-2
2. Kharabadze, N., Tsiklauri, N., Burjanadze M., Chkhaidze N (2022): Resistance of Georgian Hazelnut (Corylus L.) to Brown Marmorated Stink Bug – *Halyomorpha halys* (Stål), Journal of Nuts 10.22034/JON.2022.1958636.1171
3. Kharabadze, N. (2022): Review of the Brown Marmorated Stink Bug – *Halyomorpha halys* (Stål) in Georgia: Distribution, Biology and Management, Annals of Agrarian Science 20 (2022) 131-147.
4. Burjanadze, M., Kharabadze, N., Arjevanidze M. (2021): Bover-Ge– mycopesticedes for the control Brown Marmorated Stink Bug - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), Annals of Agrarian Science, Annals of Agrarian Science 19, 199-203 https://journals.org.ge/index.php/aans/issue/view/26
5. Burjanadze, M., Gorgadze, O., De Luca, F., Troccoli, A., Lortkipanidze, M., Kharabadze, N., Arjevanidz,e M., Fanelli, E., Tarasco, E. (2020): Potential of native entomopathogenic nematodes for the control of brown marmorated stink bug *Halyomorpha halys* in Georgia, Biocontrol Science and Technology 30:9,962-974, https://doi:10.1080/09583157.2020.1776217
6. Burjanadze, M., Natalia Kharabadze, N., Chkhidze N. (2020): Testing local isolates of entomopathogenic microorganisms against Brown Marmorated Stink Bug *Halyomorpha halys* in Georgia, BIO Web of Conferences 18, 00006, IV All-Russian Plant Protection Congress, https://doi.org/10.1051/bioconf/20201800006
7. Burjanadze, M., Kharabadze, N., Chkhaidze N. Entomopathogenic fungi and nematoodes as biocontrol agents of Brown Marmorated Stink Bug – *Halyomorpha halys* in Georgia. Book of abstracts VIII Congress on Plant Protection, p-91

Research results have been presented to international congresses and conferences:

1. Kharabadze N., Chkhaidze, N., Lobjanidze, M., Burjanadze. M. Biological agents for the control of Brown Marmorated Stink Bug (BMSB) *Halyomorpha halys* in Georgia. International Conference "Brown Marmorated Stink Bug (BMSB) Phytosanitary Regulatory Framework ". March 11-14, 2019. Tbilisi, Georgia
2. Kharabadze, N., Chkhaidze, N., Burjanadze, M., Lobjanidze, M., Didebulidze, K., Brown Marmorated Stink bug (BMSB) – *Halyomorpha halys* - a new hazard for biodiversity of Georgia. 1st International Scientific Conference. Advances and perspectives of Biodiversity Research and Conservation in Georgia. 20-22 May, 2019, Tbilisi, Georgia
3. Burjanadze, M., Kharabadze, N., Chkhaidze, N., Entomopathogenic fungi and nematoodes as biocontrol agents of brown marmorated stink bug – *Halyomorpha halys* in Georgia. Book of abstracts VIII Congress on Plant Protection, p-91. Zlatibor, Serbia, November 25-29, 2019

4. Burjanadze, M., Kharabadze, N., Arjevanidze M. “Bover-Ge – mycopesticedes for the control Brown Marmorated Stink Bug - *Halyomorpha halys* (stål) (hemiptera: pentatomidae)” 6TH Asia Pacific International Modern Sciences Congress. 2021, Deli, India
5. Burjanadze, M., Kharabadze, N., Arjevanidze, M."Effectivness of Local Isolates of Entomopathogenic Microorganisms to Brown Marmorated Stink Bug Halyomorpha halys "3rd International Modern Scientific Research Congress. May 06-08, 2022 / Istanbul Gedik University, Istanbul, Turkiye

References

მიროტაძე, მაია. *საქართველოში გავრცელებული ზოგიერთ კაკლოვანთა (თხილი) სამრეწველო ჯიშების შრობის ოპტიალური რეჟიმის დადგენა.* დისერტაცია. (თბილისი: 2011) https://dspace.nplg.gov.ge/handle/1234/166244

ნებიერიძე ელ., სავენკო რ. „*საქართველოს მწერების (უმთავრესად მავნე სახეობათა) სარკვევი,*“(თბილისი: სახელგამი, 1957) 296 გვ.

საქართველოს გარემოს დაცვისა და სოფლის მეურნეობის სამინისტრო. ახალი ამბები | საქართველოს გარემოს დაცვისა და სოფლის მეურნეობის სამინისტრო. (2019). https://mepa.gov.ge/Ge/News/Details/10602

სიჭინავა, ნინო. *თხილის ბიოლოგიური და სამეურნეო ნიშან-თვისებების შესწავლა სამეგრელოს რეგიონში.* დისერტაცია. (თბილისი: 2005) 64გვ.

ცხაკაია, ქ. მირიანაშვილი, ნ. *მცენარეთა ანატომია (პრაქტიკული კურსი).* (თბილისი: განათლება, 1954).

ხომასურიძე, (1978). *კურკოვანი, კალოვანი და სუბტროპიკული კულტურები. საქართველოს მეხილეობა.* ტომი IV. (თბილისი: მეცნერება 1978) 934 გ.

Abbott, W. S. "A Method of Computing the Effectiveness of an Insecticide." Journal of Economic Entomology 18, no. 2 (1925): 265–67. https://doi.org/10.1093/jee/18.2.265a.

Abram, P. K., J. Doyon, J. Brodeur, T. D. Gariépy, and G. Boivin. "Susceptibility of Halyomorpha Halys (Hemiptera: Pentatomidae) Eggs to Different Life Stages of Three Generalist Predators." *The Canadian Entomologist* 147, no. 2 (2014): 222–26. https://doi.org/10.4039/tce.2014.41.

Abram, Paul K., Kim A. Hoelmer, Angelita Acebes-Doria, Heather Andrews, Elizabeth H. Beers, J. Christopher Bergh, Ric Bessin, et al. "Indigenous Arthropod Natural Enemies of the Invasive Brown Marmorated Stink Bug in North America and Europe." *Journal of Pest Science* 90, no. 4 (2017): 1009–20. https://doi.org/10.1007/s10340-017-0891-7.

Acebes, A. L. "Host Plant Effects on the Biology, Behavior and Ecology of Brown Marmorated Stink Bug." Dissertation, 2016.

Acebes-Doria, Angelita L., Tracy C. Leskey, and J. Christopher Bergh. "Host Plant Effects on Halyomorpha halys (Hemiptera: Pentatomidae) Nymphal Development and Survivorship." Environmental Entomology 45, no. 3 (2016): 663–70. https://doi.org/10.1093/ee/nvw018.

Akmal, M., Freed, S., Malik, M. N., & Gul, H. T. (2013). "Efficacy of Beauveria bassiana (Deuteromycotina: Hypomycetes) against different aphid species under laboratory conditions." Pakistan Journal of Zoology 45(1): 71–78.

Arnold, K. "Halyomorpha halys (Stål, 1855), eine für die europäische Fauna neu nachgewiesene Wanzenart (Insecta: Heteroptera, Pentatomidae, Pentatominae, Cappaeini)." Mitteilungen des Thüringischen Entomologenverbandes 16(1) (2009): 19.

Aukema, B., C. Rieger, W. Rabitsch. Catalogue of the Heteroptera of the Palaearctic Region Vol. 6. Supplement. The Netherlands Entomological Society, Ede, (2013): 629 pp.

Andreadis, S. S., Gogolashvili, N. E., Fifis, G. T., Navrozidis, E. I., & Thomidis, T. "First Report of Native Parasitoids of Halyomorpha halys (Hemiptera: Pentatomidae) in Greece." Insects 12(11) (2021): 984. MDPI AG. Retrieved from http://dx.doi.org/10.3390/insects12110984.

Ayeni, A., Adeeyo, O. A., Oresegun, O. M., & Oladimeji, T. E. "Compositional Analysis of Lignocellulosic Materials: Evaluation of an Economically Viable Method Suitable for Woody and Non-woody Biomass." American Journal of Engineering Research (2015): 14-19.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., et al. "Herbivory in Global Climate Change

Research: Direct Effects of Rising Temperature on Insect Herbivores." Global Change Biology (2002), 8: 1–16. https://doi.org/10.1046/j.1365-2486.2002.00451.x.

Bariselli, M., Bugiani, R., and Maistrello, L. "Distribution and Damage Caused by Halyomorpha halys in Italy." EPPO Bulletin (2016) 0 (0): 1–3. ISSN 0250-8052. DOI: 10.1111/epp.12289.

Battiston, R., Massa, B. "The Mantids of Caucasus (Insecta Mantodea)." *Atti dell'Accademia Roveretana degli Agiati* (2008), VIII(B): 5–28.

Beraia, I., Khabeishvili, V., Tavdumadze, K. "Subtropical Industrial Crops." 1984. 401 p. (in Georgian).

Bergmann, E., Bernhard, K. M., Bernon, G., Bickerton, M., Gill, S., Gonzales, C., Hamilton, G. C., Hedstrom, C., Kamminga, K., Koplinka-Loehr, C., et al. "Host Plants of the Brown Marmorated Stink Bug." (2015). http://www.stopbmsb.org/where-is-bmsb/host-plants/ (last accessed on 28 December 2018).

Bergmann, E., Venugopal, P. D., Martinson, H. M., Raupp, M. J., Shrewsbury, P. M. "Host Plant Use by the Invasive Halyomorpha halys (Stål) on Woody Ornamental Trees and Shrubs." PLoS One (2016), 11(2): e0149975. https://doi.org/10.1371/journal.pone.0149975.

Bernon, G. "Biology of Halyomorpha halys, the Brown Marmorated Stink Bug (BMSB)." Final Report – USDA APHIS CPHST (2004): 17 pp.

Biddinger, David J., Alex Surcică, and Neelendra K. Joshi. "A Native Predator Utilising the Invasive Brown Marmorated Stink Bug, Halyomorpha Halys (Hemiptera: Pentatomidae) as a Food Source." Biocontrol Science and Technology 27, no. 7 (2017): 903–7. https://doi.org/10.1080/09583157.2017.1354247.

Bidochka, Michael J., and George G. Khachatourians. "Protein Hydrolysis in Grasshopper Cuticles by Entomopathogenic Fungal Extracellular Proteases." Journal of Invertebrate Pathology 63, no. 1 (1994): 7–13. https://doi.org/10.1006/jipa.1994.1002.

Birah, A., Chilana, P., Shukla, U. K., and Gupta, G. P. "Mass Rearing of Greater Wax Moth (Galleria mellonella L.) on Artificial Diet." Indian Journal of Entomology (2008), 70: 389-392.

Blaauw, B. R., Polk, D., Nielsen, A. L. "IPM-CPR for Peaches: Incorporating Behaviorally-Based Methods to Manage Halyomorpha halys and Key Pests in Peach." Pest Management Science (2015) 71: 1513–1522.

Bosco, L., Moraglio, S. T., & Tavella, L. "Halyomorpha halys, a Serious Threat for Hazelnut in Newly Invaded Areas." Journal of Pest Science (2018) 91: 661–670.

Boudet, A. "Lignins and Lignification. In: Selected Issues." Plant Physiology and Biochemistry (2000). https://doi:10.1016/s0981-9428(00)00166-2.

Brandfass, Ch., and P. Karlovsky. "Upscaled CTAB-Based DNA Extraction and Real-Time PCR Assays for Fusarium culmorum and F. graminearum DNA in Plant Material with Reduced Sampling Error." International Journal of Molecular Sciences 9, no. (2008) 11: 2306-2321. https://doi.org/10.3390/ijms9112306.

Brannoch, S. K., F. Wieland, J. Rivera, K.-D. Klass, O. Béthoux. "Svenson GJ Manual of Praying Mantid Morphology, Nomenclature, and Practices (Insecta, Mantodea)." ZooKeys (2017) 696: 1–100. https://doi.org/10.3897/zookeys.696.12542

Brundtland, G. 1987. Report of the World Commission on Environment and Development: Our Common Future. United Nations General Assembly Document A/42/427.

Burjanadze, M., S. Jaronski, K. Koridze, A. Supatashvili. 2018. "Diversity of Entomopathogenic Fungi from Kintrishi National Area Forest Ecosystem of Georgia." Invertebrate Pathology and Microbial Control and the 51st Annual Meeting of the Society for Invertebrate Pathology, 12th - 16th August, Gold Coast, Australia.

Burjanadze, M., O. Gorgadze, F. De Luca, A. Troccoli, M. Lortkipanidze, N. Kharabadze, M. Arjevanidze, E. Fanelli, E. Tarasco. 2020. "Potential of Native Entomopathogenic Nematodes for the Control of Brown Marmorated Stink Bug Halyomorpha halys in Georgia." Biocontrol Science and Technology (2020). https://doi.org/10.1080/09583157.2020.1776217

Burjanadze, M., N. Kharabadze, M. Arjevanidze. 2021. "Bover-Ge – Mycopesticides for the Control of Brown Marmorated Stink Bug - Halyomorpha halys (Stål) (Hemiptera: Pentatomidae)." Annals of Agrarian Science v. 19, no. 3, pp 199-204.

Burjanadze, M., N. Kharabadze, N. Chkhaidze. "Entomopathogenic Fungi and Nematodes as Biocontrol Agents of Brown Marmorated Stink Bug – Halyomorpha halys in Georgia." Book of Abstracts VIII Congress on Plant Protection, p-91. Zlatibor, Serbia, November 2019, 25-29.

Burjanadze, M., N. Kharabadze, N. Chkhidze. "Testing Local Isolates of Entomopathogenic Microorganisms against Brown Marmorated Stink Bug Halyomorpha halys in Georgia." BIO Web of Conferences 18 (2020): 00006. doi: 10.1051/bioconf/20201800006

Butt, T. M., and M. S. Goettel. "Bioessays of Entomopathogenous Fungi." In Bioessays of Entomopathogenic Microbes and Nematodes, edited by A. Navon and K. R. S. Ascher, 141–191. Wallingford, Oxon, UK: CAB International, 2000.

Callot, H., and C. Brua. "Halyomorpha halys (Stål, 1855), la Punaise diabolique, nouvelle espèce pour la faune de France (Heteroptera Pentatomidae)." L'Entomologiste 69, no. 2 (2013): 69–71.

Çerçi, B., and Ö. Koçak. "Further Contribution to the Heteroptera (Hemiptera) Fauna of Turkiye with a New Synonymy." Acta Biologica Turcica 30, no. 4 (2017): 121–127.

Chaparro-Encinas, L. A., G. L. Arellano-Wattenbarger, F. I. Parra-Cota, et al. "A Modified CTAB and Trizol® Protocol for High-Quality RNA Extraction from Whole Wheat Seedlings, Including Rhizosphere." CEREAL RESEARCH COMMUNICATIONS 48 (2020): 275–282. doi: 10.1007/s42976-020-00046-9

Cheong, P., T. R. Glare, M. Rostás, and S. R. Haines. "Microbial-Based Biopesticides." In Microbial-Based Biopesticides, 177–189. Springer, 2016.

Choi, D. S., K. C. Kim, and K. C. Lim. 2000. "The Status of Spot Damage and Fruit Piercing Pests on Yuzu (Citrus junos) Fruit." *Korean J. Appl. Entomol.* 39: 259–266.

Chung, B. K., S. W. Kang, and J. H. Kwon. 1995. "Damages, Occurrences and Control of Hemipterous Insects in Nonastringent Persimmon Orchards." J. Agric. Sci. 37: 376–382.

Cianferoni, F., F. Graziani, P. Dioli, and F. Ceccolini. 2018. "Review of the Occurrence of Halyomorpha halys (Hemiptera: Heteroptera: Pentatomidae) in Italy, with an Update of Its European and World Distribution." Biologia, 1–9.

Ciceoi, Roxana, I.-G. Bolocan, and Ionela Dobrin. December 2017. "The Spread of Brown Marmorated Stink Bug, Halyomorpha halys, in Romania." JOURNAL of Horticulture, Forestry and Biotechnology 21, no. 3 (2017): 15–20.

Cira, T. M., R. C. Vennette, J. Aigner, T. Kuhar, D. E. Mullins, S. E. Gabbert, and W. D. Hutchison. 2015. "Cold Tolerance of Halyomorpha halys (Hemiptera: Pentatomidae) across Geographic and Temporal Scales." Environ. Entomol. DOI: 10.1093/ee/nvv220

Copeland, J., and A. D. Carlson. 1979. "Prey Capture in Mantids: A Non-stereotyped Component of Lunge." J Insect Physiol 25:263–269. https://doi.org/10.1016/0022-1910(79)90053-2

Copping, L. G. 2009. The Manual of Biocontrol Agents. British Crop Production Council. United Kingdom. Technology & Engineering - 851 pages.

Cusumano, A., Peri, E., Amodeo, V., McNeil, J. N., & Colazza, S. (2013). "Intraguild Interactions between Egg Parasitoids: Window of Opportunity and Fitness Costs for a Facultative Hyperparasitoid." PLoS One, 8:e64768.

Dhawan, M., & Joshi, N. (2017). "Enzymatic Comparison and Mortality of Beauveria bassiana against Cabbage Caterpillar Pieris brassicae LINN." Braz J Microbiol, 48(3), 522-529. doi: 10.1016/j.bjm.2016.08.004.

Dioli, P., Leo, P., & Maistrello, L. (2016). "Prime Segnalazioni in Spagna e in Sardegna della Specie Aliena Halyomorpha halys (Stål, 1855) e Note sulla Sua Distribuzione in Europa (Hemiptera, Pentatomidae)." Rev gad Entom, 7(1), 539–548.

Doo-Hyung Lee et al. (2013). "Review of the Biology, Ecology, and Management of Halyomorpha halys (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea." Environmental Entomology, 42(4), 627–641. https://doi.org/10.1603/EN13006.

Drakhovskaia, M. (1962). Prognosis of Plant Protection. Moscow, Nauka. Pp-158.

Dumbadze, G., Gokturk T., Jgenti, L., & Chelidze, N. (2019). "Distribution of Brown Marmorated Stink Bug (Halyomorpha halys), Bioecological Features and Control Mechanisms in Batumi (Georgia)." SETSCI Conference Proceedings, 4(6), 539-542.

Duthie, C. (2012). "Risk Analysis of Halyomorpha halys (Brown Marmorated Stink Bug) on all Pathways." Ministry for Primary Industries, Wellington. 51 pp.

EPPO Global Database (2018). URL: https://gd.eppo.int (last update: 29 April 2018).

EPPO Global Database. "Halyomorpha halys (HALYHA) [World Distribution]." EPPO Global Database. Retrieved June 19, 2022, from https://gd.eppo.int/taxon/HALYHA/distribution.

Eskin, N. B., & Bibin, A. R. "Focus of the Box Tree Moth in a Yew-Box Grove." Kavkaz zapovednyi, 2014, N 8 (124), P. 7. (In Russian).

Evlakhova, A. A. (1974). Entomopathogenic Fungi. Classification, Biology, Practical Significance. Leningrad, Russia: Nauka Press. pp. 1-260.

Faria, M., Lopes, R. B., Souza, D. A., & Wraight, S. P. (2015). "Conidial Vigor vs. Viability as Predictors of Virulence of Entomopathogenic Fungi." J. Invertebr. Pathol., 125, 68–72.

Faúndez, E. I., & Rider, D. A. (2017). "The Brown Marmorated Stink Bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) in Chile." Arquivos Entomolóxicos, 17, 305–307.

Field, S. A. (1998). "Patch Exploitation, Patch-Leaving and Pre-Emptive Patch Defence in the Parasitoid Wasp Trissolcus basalis (Insecta: Scelionidae)." Ethology, 104, 323–338.

Formella, A. J. (2019). Ecology of the Brown Marmorated Stink Bug (Hemiptera: Pentitomidae): Oviposition on Different Trees, Nymphal Development on Various Vegetable Hosts, and Assessment of a Bacterial Pathogen (Doctoral dissertation, Virginia Tech) [http://hdl.handle.net/10919/99202](http://hdl.handle.net/10919/99202

Francati, S., Masetti, A., Martinelli, R., Mirandola, D., Anteghini, G., Busi, R., & Dindo, M. L. (2021). "Halyomorpha halys (Hemiptera: Pentatomidae) on kiwifruit in northern Italy:

Phenology, infestation, and natural enemies assessment." Journal of Economic Entomology, 114(4), 1733-1742.

Franz, J. M. (1968). "Zur Berechnung Des Wirkungsgrades Einer Mikrobiologischen Bekämpfung von Schadinsekten." *Anzeiger für Schäadlingskunde*, 41(5), 65–71. https://doi.org/10.1007/bf02329909.

Funayama, K. (1996). "Sucking Injury on Apple Fruit by the Adult of Brown Marmorated Stinkbug Halyomorpha mista (Uhler)." Annu. Rep. Plant Prot. North. Jpn., 47, 140–142.

Funayama, K. (2002). "Comparison of the Susceptibility to Injury of Apple Cultivars by Stink Bugs." Jpn. J. Appl. Entomol. Zool., 46, 37–40.

Gadalla, S. M. (2004). "New Records of Pentatomomorpha (Hemiptera) from Egypt." Journal of Union of Arab Biologists, A, Zoology, 21, 43–58.

Gagnidze, I. R., Nakhutsrishvili, G., & Maier, G. (2005). Vascular Plants of Georgia: a Nomenclatural Checklist. Georgian Academy of Sciences, pp. 243.

Gapon, D. A. (2016). "First Records of the Brown Marmorated Stink Bug Halyomorpha halys (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia." Entomol Rev, 96(8), 1086–1088. https://doi.org/10.1134/S001387381608011X.

Garrouste, R., Nel, P., Nel, A., Horellou, A., & Pluot-Sigwalt, D. (2015). "Halyomorpha halys (Stål 1855) en Île de France (Hemiptera: Pentatomidae: Pentatominae): Surveillons la punaise diabolique." Ann Soc Entomol Fr (NS), 50(3–4)(2014), 257–259. https://doi.org/10.1080/00379271.2014.990250.

Gilbert, J. D. J. (2011). "Insect Dry Weight: Shortcut to a Difficult Quantity Using Museum Specimens." Fla Entomol, 94, 964–970.

Glazer, I., & Lewis, E. E. (2000). "Bioassays for Entomopathogenic Nematodes." In A. Navon & K. R. S. Ascher (Eds.), Bioassay for Entomopathogenic Microbes and Nematodes (pp. 229–247). CAB International.

Gorgadze, O., Bakhtadze, G., Kereselidze, M., & Lortkhipanidze, M. (2017). "The Efficacy of Entomopathogenic Agents against Halyomorpha halys (Hemiptera: Pentatomidae)." *International Journal of Current Research*, 9(12), 62177-62180.

Gorgadze, O.A., and M.G. Lortkipanidze. 2006. "Nematoda Steinernema gurgistana sp. n. (Rhabditida: Steinernematidae) from Agriotes gurgistana F." *Bulletin of the Georgian National Academy of Sciences* 174: 117-122.

Gorgadze, O., Fanelli, E., Lortkhipanidze, M., Troccoli, A., Burjanadze, M., Tarasco, E., & De Luca, F. (2018). "Steinernema borjomiense n. Sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Georgia." BRILL. Nematology, 20(7), 653–669. https://doi.org/10.1163/15685411-00003167.

Gouli, V., Gouli S, Skinner M., Hamilton G., Kim JS, Parker B. (2012) "Virulence of select entomopathogenic fungi to the brown marmorated stink bug, Halyomorpha halys (Stål) (Heteroptera: Pentatomidae)," Pest Management Sciences, v.,68(2), 155-157.

Grazia, J., Panizzi, A.R., Greve, C., Schwertner, C.F., Campos, L.A., Garbelotto, T. de A., et al., 2015. "Stink Bugs (Pentatomidae)." In: True Bugs (Heteroptera) of the Neotropics, pp. 681–756. Springer, Dordrecht.

Güncan, A.; Gümüş, E. (2019). "Brown marmorated stink bug, Halyomorpha halys (Stål, 1855) (Hemiptera: Heteroptera, Pentatomidae), a new and important pest in Turkiye." Journal of Entomological News, 128(2), 204-210.

Hajek, A. E., Solter, L. F., Maddox, J. V., Huang, W.-F., Estep, A. S., Krawczyk, G., Weber, D. C., Hoelmer, K. A., Sanscrainte, N. D., & Becnel, J. J. (2018). "Nosema maddoxi sp. Nov. (microsporidia, Nosematidae), a widespread pathogen of the green stink bug Chinavia hilaris (Say) and the brown marmorated stink bug Halyomorpha halys (Stål)." Journal of Eukaryotic Microbiology, 65(3), 315–330. https://doi.org/10.1111/jeu.12475.

Hall, T.A. (1999) "BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT." Nucleic Acids Symposium Series, 41, 95-98.

Hano C, Addi M, Bensaddek L, Crônier D, Baltora-Rosset S, Doussot J, Lamblin F (2005) "Differential accumulation of monolignol-derived compounds in elicited flax (Linum usitatissimum) cell suspension cultures." *Planta*, 975-989. https://doi:10.1007/s00425-005-0156-1.

Harz K & Kaltenbach A (1976) "Die Orthopteren Europas 3." - Dr. W. Junk B.V. The Hague, Vol 3, 129-169.

Haye T, Gariepy T, Hoelmer K, Rossi JP, Streito JC, Tassus X, Desneux N (2015 b) "Range expansion of the invasive brown marmorated stinkbug, Halyomorpha halys: an increasing threat to field, fruit and vegetable crops worldwide." Journal of Pest Science, 88, 665–673. https://doi.org/10.1007/s10340-015-0670-2.

Haye, T., Wyniger, D., & Gariepy, T. D. (2014). "Recent range expansion of brown marmorated stink bug in Europe." In: Müller G, Pospichil R, Robinson W (eds) Proceedings of the eighth international conference on urban pests, Veszprém, Hungary, pp 309–314.

Haye, T., Fischer, S., Zhang, J., & Gariepy, T. (2015). "Can native egg parasitoids adopt the invasive brown marmorated stink bug, Halyomorpha halys (Heteroptera: Pentatomidae), in Europe?" *Journal of Pest Science*, 88, 693–705.

Hayward, J. (1990). "Agricultural extension: The World Bank's experience and approaches." In FAO Report of the Global Consultation on Agricultural Extension (p. 115-134). Rome: FAO.

Heckmann, R. (2012). "Erster Nachweis von Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) für Deutschland." Heteropteron 36, 17–18.

Hedstrom, C. S., Shearer, P. W., Miller, J. C., Walton, V. M. (2014). "The Effects of Kernel Feeding by Halyomorpha halys (Hemiptera: Pentatomidae) on Commercial Hazelnuts." Journal of Economic Entomology, 107(5), 1858–1865. https://doi.org/10.1603/EC14263.

Hemala, V., & Kment, P. (2017). "First record of Halyomorpha halys and mass occurrence of Nezara viridula in Slovakia." Plant Prot Sci, 4, 247–253. https://doi.org/10.17221/166/2016-pps.

Herlihy, M. V., Talamas, E. J., Weber, D. C. (2016). "Attack and Success of Native and Exotic Parasitoids on Eggs of Halyomorpha halys in Three Maryland Habitats." PLOS ONE, 11(3): e0150275. https://doi.org/10.1371/journal.pone.0150275.

Hoebeke, E. R., & Carter, E. M. (2003). "Halyomorpha halys (Sta˚l) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America." Proc Entomol Soc Wash, 105, 225–237.

Hoffman, W. E. (1931). "A pentatomid pest of growing beans in South China." Peking Nat. Hist. Bull, 5(3), 25-26.

Holling, C. S. (1964). "The analysis of complex population processes." Can. Entomol, 96(1–2), 335–347. https://doi.org/10.4039/Ent96335-1.

Humber, R. (1997). "Fungi Identification." In: Manual of Techniques in Insect Pathology (1st ed.), Chapter V-1, San Diego, CA, USA, 153-185.

IBM Corp. (2015). "IBM SPSS Statistics for Windows, Version 23.0." Armonk, NY: IBM Corp.

Inglis, G.D., J. Enkerli, M.S. Goettel (2012). "Laboratory techniques used for entomopathogenic fungi: Hypocreales." In: Manual of Techniques in Invertebrate Pathology, Chapter VII, 2nd ed., Great Britain: 149-253.

Inkley, D. B. (2012). "Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae)." J. Entomol. Sci., 47, 125–130.

Iwasaki, T. (1991). "Predatory behavior of the praying mantid, Tenodera aridifolia II. Combined effect of prey size and predator size on the prey recognition." J Ethol, 9, 77–81. https://doi.org/10.1007/BF02350211.

Jakely, E., & Nikolashvili, A. (2019). "The results of bioecological study of brown marmorated stink bug - Halyomorpha halys (Stal) in the conditions of Western Georgia." Georgian Academy of Agricultural Sciences Bulletin, N1 (41), 70-75.

Japaridze, I. (1958). "Workshop on Chemistry microscopic plants." Moscow: Soviet science, (in Russian)

Japoshvili, G. T., Arabuli, T., Salakaia, M., Tskaruashvili, Z., Kirkitadze, G., Talamas, E. (2022). "Surveys for Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) and its biocontrol potential by parasitic wasps in the Republic of Georgia (Sakartvelo)." *Phytoparasitica*, 1-11. https://doi.org/10.1007/s12600-021-00949-1.

Jentsch, P. (Winter 2017). "Expanding the Range of the Samurai Wasp, Trissolcus japonicus, in New York Orchards" (PDF). Fruit Quarterly, 25(4). Retrieved December 3, 2019.

Jentsch, P. J. (March 13, 2019). "Citizen Science Efforts for Redistribution of Samurai Wasp in NYS." Cornell University, Department of Entomology, Hudson Valley Laboratory. Retrieved December 3, 2019.

Jesús, L. R., Kamiyama, M. T., Hernandez, G., Nay, J., Hoddle, M. S. (2017). "Assessment of Feeding Acceptance and Injury of Kerman Pistachios, Pistacia Vera, by Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)." Journal of Insect Science, 17(5). https://doi.org/10.1093/jisesa/iex084.

Johnson, N. (1984a). "Systematics of Nearctic Telenomus: classification and revisions of the podisi and phymatae groups (Hymenoptera: Scelionidae)." Bulletin of the Ohio Biological Survey, 6(3), 1-113.

Johnson, N. (1984b). "Revision of the Nearctic species of the Trissolcus flavipes group (Hymenoptera: Scelionidae)." Proceedings of the Entomological Society of Washington, 86, 797-807.

Johnson, N. (1985). "Systematics of New World Trissolcus (Hymenoptera: Scelionidae) species related to T. basalis." The Canadian Entomologist, 117, 431-445.

Johnson, N. (1987a). "Systematics of New World Trissolcus, a genus of pentatomid egg-parasites (Hymenoptera: Scelionidae): Neotropical species of the flavipes group." Journal of Natural History, 21, 285-304.

Johnson, N. (1987b). "The Neotropical telenomine genus Phanuropsis Girault (Hymenoptera: Scelionidae)." Annals of the Entomological Society of America, 80, 660-663.

Jones, A. L. (2013). "Indigenous natural enemies of the invasive brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae)." Master of Science, College Park, Department of Entomology, University of Maryland.

Jones, A. L., Jennings, D. E., Hooks, C. R. R., Shrewsbury, P. M. (2014). "Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, Halyomorpha halys." Biol. Control, 78, 61–66. DOI: 10.1016/ Journal of Biocontrol, http://dx.doi.org/10.1016/j.biocontrol.2014.07.011.

Jones, J. R., and Lambdin, P. L. (2009). "New county and state records for Tennessee of an exotic pest, Halyomorpha halys (Hemiptera: Pentatomidae), with potential economic and ecological implications." Florida Entomol., 92, 177-179.

Josef, S. V., Stallings, J. V., Leskey, T. C., Krawczyk, G., Polk, D., Butler, B., Bergh, J. C. (2014). "Spatial distribution of brown marmorated stink bug (Hemiptera: Pentatomidae) injury at harvest in Mid-Atlantic apple orchards." J Econ Entomol, 105, 1839-1848.

Josifov, M. V., & Kerzhner, I. M. (1978). "Heteroptera aus Korea. II. Teil (Aradidae, Berytidae, Lygaeidae, Pyrrhocoridae, Rhopalidae, Alydidae, Coreidae, Urostylidae, Acanthosomatidae, Scutelleridae, Pentatomidae, Cydnidae, Plataspidae)." Fragm.faun., 23(9), 137-196.

Kagen, S. L. (1990). "Inhalant allergy to arthropods." Clinical Reviews in Allergy, 8, 99–125. https://doi.org/10.1007/BF02914439.

Karpun, N. N., Protsenko, V. E. (2016). "Marmorated bug (Halyomorpha halys Stål.) appeared in humid subtropics of Russia and Abkhazia." Federal State Budgetary Institution "All-Russian Scientific Research Institute Floriculture and subtropical crops." http://vniisubtrop.ru/novosti/769-mramornyj-klop-halyomorpha-halys-st-l-poyavilsya-vo-vlazhnykh-subtropikakh-rossii-i-abkhazii.html (in Russian).

Kereselidze, M., Pilarska, D., & Linde, A. (2019). "First record of a microsporidium in the population of brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) in the Republic of Georgia." Acta Zoologica Bulgarica, 71(3), 427-432.

Kereselidze, M., Aleksidze, G., & Haye, T. (2018). "First record native parasitoid attacking Halyomorpha halys (Heteroptera: Pentatomidae) in Georgia." Bulletin. Georgian Academy of Agricultural Science, 39, 127–129.

Kereselidze, Manana; Pilarska, Daniela; Linde, Andreas; Sanscrainte, Neil D. & Hajek, Ann E. (2020). "Nosema maddoxi infecting the brown marmorated Stink bug, Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), in the Republic of Georgia." Biocontrol Science and Technology. DOI: 10.1080/09583157.2020.1787346.

Khadka, A., Hodges, A. C., Leppla, N. C., & Tillman, P. G. (2020). "The effects of relative humidity on Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) egg hatch, nymph survival, and adult reproduction." The Florida Entomologist, 103(1), 136–138. https://www.jstor.org/stable/48610645.

Kharabadze, N., Tsiklauri, N., Burjanadze, M., & Chkhaidze, N. (2022). "Resistance of Georgian Hazelnut (Corylus L.) to Brown Marmorated Stink Bug – Halyomorpha halys (Stål)." Journal of Nuts. https://doi.org/10.22034/JON.2022.1958636.1171.

Kharabadze, N., Chkhaidze, N., Lobjanidze, M., & Burjanadze, M. (2019). "Biological agents for the control of Brown Marmorated Stink Bug (BMSB) Halyomorpha halys in Georgia." International Conference "Brown Marmorated Stink Bug (BMSB) Phytosanitary Regulatory Framework". March 11-14, 2019. Tbilisi, Georgia.

Kharabadze, N., Chkhaidze, N., Abramishvili, T., et al. (2022). "First report of Hierodula transcaucasica (Brunner von Wattenwyl, 1878) predation on the Halyomorpha halys (Stål, 1855) in Georgia." Int J Trop Insect Sci. https://doi.org/10.1007/s42690-022-00826-2.

Kharabadze N. (2019) Natural Enemies as Potential Biological Agents for the Control of Brown Marmorated Stink Bug (H. halys) - *Halyomorpha halys* in Georgia. International conference on Brown Marmorated Stink Bug (*H. halys*)-Phytosanitary Regulator Framework. Tbilisi, Republic of Georgia. 11-14 March. Tbilisi.

Kharebava, M. (1967). Ecology of Subtropical Culturs. 207 p. (in Georgian).

Khomasuridze, T. (1978). Stone-fruits, Fruits, Nuts and Subtropical Crops (in Georgian). Georgian Fruits. Volume IV. (Tbilisi: Metsniereba, 1978) 934pp.

Khorosheva, T. M., Chekmarova, L. I. (2013). Plant Immunity: short lecture courses for graduate students. 69 p. (in Russian).

Khrimian, A., Shearer, P. W., Zhang, A., Hamilton, G., Aldrich JR. (2008). "Field trapping of the invasive brown marmorated stink bug, Halyomorpha halys, with geometric isomers of methyl 2,4,6-decatrienoate." J Agric. Food Chem., 56(197–203).

Khrimian, A., Zhang, A., Weber, D. C., Ho, H. Y., Aldrich, J. R., et al. (2014). "Discovery of the aggregation pheromone of the brown marmorated stink bug (Halyomorpha halys) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols." J. Nat. Prod., 77(1708–17).

Kistner, E. J. (2017). "Climate Change Impacts on the Potential Distribution and Abundance of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) with Special Reference to North America and Europe." Environ. Entomol., 46, 1212–1224. https://doi.org/10.1093/ee/nvx157.

Kobayashi, T., and S. Kimura. (1969). "The studies on the biology and control of house-entering stink bugs. Part 1. The actual state of the hibernation of stink bugs in houses." Bull. Tohoku Natl. Agric. Exp. St. Morioka, 37, 123Ð138.

Konopka, J. K., Haye, T., Gariepy, T., Mason, P., Gillespie, D., McNeil, J. N. (2017). "An exotic parasitoid provides an invasional lifeline for native parasitoids." Ecol. Evol., 7, 277–84.

Kriticos, D. J., Kean, J. M., Phillips, C. B., Senay, S. D., Acosta, H., Haye, T. (2017). "The potential global distribution of the brown marmorated stink bug, Halyomorpha halys, a critical threat to plant biosecurity." J Pest Sci., 90, 1033–1043. https://doi.org/10.1007/s10340-017-0869-5.

Kuhar, T. P., Doughty, H., Kamminga, K., Lilliston, L. (2012). "Evaluation of insecticides using bean dip bioassay for control of brown marmorated stink bug, 2011." Arthropod Manag. Tests., 37. doi:10.4182/amt.2012.E41.

Kuhar, T. P., Doughty, H., Kamminga, K., Wallingford, A., Philips, C., Aigner, J. (2012a). "Evaluation of insecticides for the control of brown marmorated stink bug in bell peppers in Virginia experiment 1, 2011." Arthropod Manag. Tests., 37. E37doi:10.4182/amt.2012.E37

Kuhar, T. P., Doughty, H., Kamminga, K., Wallingford, A., Philips, C., Aigner, J. (2012b). "Evaluation of insecticides for the control of brown marmorated stink bug in bell peppers in Virginia experiment 2, 2011." Arthropod Manag. Tests., 37. E38doi: 10.4182/amt.2012.E38.

Kuhar, T. P., Doughty, H., Kamminga, K., Wallingford, A., Philips, C., Aigner, J. (2012c). "Evaluation of insecticides for the control of brown marmorated stink bug in bell peppers

in Virginia experiment 3, 2011." Arthropod Manag. Tests., 37. E39doi:10.4182/amt.2012.E39.

Kuhar, T. P., Doughty, H., Kamminga, K., Wallingford, A., Philips, C., Aigner, J. (2012d). "Evaluation of insecticides for the control of brown marmorated stink bug in bell peppers in Virginia experiment 4, 2011." Arthropod Manag. Tests., 37. E40doi:10.4182/amt.2012.E40.

Kuhar, T. P., Kamminga, K. (2017). "Review of the chemical control research on Halyomorpha halys in the USA." J Pest Sci, 90, 1021–1031. https://doi.org/10.1007/s10340-017-0859-7.

Kunelauri, N., Baramidze, V., Mikeladze, E., Shubladze, E., Koridze, K., Kharabadze, N., Burjanadze, M. (2019). "Genotype diversity and pathogenicity of Beauveria spp. Isolates from different ecoregions of Georgia." Annals of Agrarian Science, 17, pp.82–101.

Lacey, L. A. (ed.) (1997). Manual of Techniques in Insect Pathology. Academic Press, London.

Lacey, L. A., & Kaya, H. K. (2007). Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests. Springer.

Lal, R. (2008). "Soils and sustainable agriculture. A review." Agron. Sustain. Dev., 28, 57–64. DOI:10.1051/agro:2007025.

Lauvergeat, V., Lacomme, C., Lacombe, E., Lasserre, E., Roby, D., Grima-Pettenati, J. (2001). "Two cinnamoyl-CoA reductase (CCR) genes from Arabidopsis thaliana are differentially expressed during development and in response to infection with pathogenic bacteria." Phytochemistry, 57(7), 1187–1195. https://doi:10.1016/s0031-9422(01)00053-x.

Lee, D. H., Short, B. D., Joseph, S. V., Bergh, J. C., Leskey, T. C. (2013). "Review of the biology, ecology, and management of Halyomorpha halys (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea." Environ. Entomol., 42, 627–641. https://doi.org/10.1603/en1300.

Lee, D.-H., Leskey, T. C. (2015). "Flight behavior of foraging and overwintering brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae)." Bulletin of Entomological Research, 105(5), 566–573. https://doi.org/10.1017/s0007485315000462.

Lee, D.-H., A. L. Nielsen, and T. C. Leskey. (2014). "Dispersal capacity and behavior of nymphal stages of Halyomorpha halys (Hemiptera: Pentatomidae) evaluated under laboratory and field conditions." J. Insect Behav., 27, 639-651.

Leskey, T. C., G. C. Hamilton, A. L. Nielsen et al., (2012). "Pest status of the brown marmorated stink bug, Halyomorpha halys in the USA." Outlooks Pest Manag., 23(5), 218-226.

Leskey TC, Lee DH, Short BD, Wright SE. (2012). "Impact of insecticides on the invasive Halyomorpha halys (Hemiptera: Pentatomidae): analysis of insecticide lethality." J Econ Entomol., 105, 1726–1735. doi:10.1603/EC12096.

Leskey TC, Nielsen AL (2018) "Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management." Annu Rev Entomol., 63, 599–618. https://doi.org/10.1146/annurev-ento-020117-043226.

Leskey, T. C. (2015). "Flight behavior of foraging and overwintering brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae)." Bull. Entomol. Res., 105, 566–73.

Lichtfouse, E. (2013). "Climate Change, Intercropping, Pest control and Beneficial Microorganisms." Sustainable Agriculture Reviews, 1-9.

Lin, S. Y., Dence, C. W. (1992). "The determination of lignin." In: Methods in Lignin Chemistry, by C.W. Dence, 33-61. Heidelberg: Springer-Verlag.

Liu, Q., Luo, L., & Zheng, L. (2018). "Lignins: Biosynthesis and Biological Functions in Plants." International Journal of Molecular Sciences, 19(2), 335. https://doi.org/10.3390/ijms19020335.

Macavei, L. I., Bâeþan, R., Oltean, I., Florian, T., Varga, M., Costi, E., Maistrello, L. (2015). "First detection of Halyomorpha halys Stål, a new invasive species with a high potential for damage to agricultural crops in Romania." Lucrãri ªtiinþifice seria Agronomie, 58(1), 105-108.

Mahr, D. L., Whitaker, P., Ridgway, N. M. (2008). "Biological control of insects and mites: An introduction to beneficial natural enemies and their use in pest management." University of Wisconsin Cooperative Extension, No. A3842, Madison, WI.

Maistrello, L., Dioli, P., Vaccari, G., Nannini, R., Bortolotti, P., Caruso, S., Costi, E., Montermini, A., Casoli, L., Bariselli, M. (2014). "Primi rinvenimenti in Italia della cimice esotica Halyomorpha halys, una nuova minaccia per la frutticoltura." Atti Giornate Fitopatologiche, 1, 283–288.

Maldonado, H., Benko, M., Isern, M. (1970). "Study of the role of binocular vision in mantids to estimate long distances, using the deimatic reaction as an experimental situation." Z Vergl Physiol, 68, 72–83. https://doi.org/10.1007/BF00297813.

Maldonado, H., Rodriguez, E. (1971). "Depth perception in the praying mantid." Physiol Behay, 8, 751–759. https://doi.org/10.1016/0031-9384(72)90107-2.

Malumphy, C. (2014). "Second interception of Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) in Britain." Het News, 21, 4–5.

Mamedov, Z. M. (2018). "Brown marmorated sting bug (Halyomorpha halys Stål.) the pest of orchard, forest and decorative trees in Azerbaijan." Georgian Academy of Agricultural Sciences Bulletin, N2 (40), 88-89.

Martinson, H. M., Venugopal, P. D., Bergmann, E. J., Shrewsbury, P. M., Raupp, M. J. (2015). "Fruit availability influences the seasonal abundance of invasive stink bugs in ornamental tree nurseries." J Pest Sci., 88, 461–468. doi: 10.1007/s10340-015-0677-8.

Masters, G., Norgrove, L. (2010). "Climate change and invasive alien species." CABI Working Paper 1, 30 pp.

Mathews, C. R., Blaauw, B., Dively, G., Kotcon, J., Moore, J., Ogburn, E., Pfeiffer, D. G., Trope, T., Walgenbach, J. F., Welty, C., Zinati, G., Nielsen, A. L. (2017). "Evaluating a polyculture trap crop for organic management of Halyomorpha halys and native stink bugs in peppers." J Pest Sci. doi:10.1007/s10340-017-0838-z.

Matsura T, Inoue T, Hosomi Y. 1975. "Ecological studies of a mantid, Paratenodera angustipennis de Saussure. I. Evaluation of the feeding condition in natural habitats." Res Pop Eco 17:64–76.

Matsura T, Yoshimaya H, Nagai T. 1984. "Growth, prey consumption and food assimilation efficiency in a mantid, Paratenodera angustipennis (S.)." Kontyu (Tokyo) 52:37–49.

Maurel J-P, Blaye G, Valladares L, Roinel É, Cochard PO. 2016. "Halyomorpha halys (Stål, 1855), la punaise diabolique en France, à Toulouse (Heteroptera; Pentatomidae)." Carnets Natures 3:21–25.

Mazzoni V, Eriksson A, Anfora G, et al. 2014. "Active space and the role of amplitude in plant-borne vibrational communication." Springer, Berlin, pp 125–145. DOI: 10.1007/978-3-662-43607-3_8.

Mazzoni V, Lucchi A, ˇ Cokl A, Prešern J, and Virant-Doberlet M. 2009. "Disruption of the reproductive behavior of Scaphoideus titanus by playback of vibrational signals." Entomol Exp Appl 133:174–185.

Mazzoni V, Polajnar J, Baldini M, Valerio M, Stacconi R, Anfora G, Guidetti R, Maistrello L. 2017. "Use of Substrate-Borne Vibrational Signals to Attract the Brown Marmorated Stink Bug, Halyomorpha Halys." Journal of Pest Science 90, no. 4 (2017): 1219–29. DOI: 10.1007/s10340-017-0862-z.

Mertz TL, Jacobs SB, Craig TJ, Ishmael FT. 2012. "The brown marmorated stinkbug as a new aeroallergen." Journal of Allergy and Clinical Immunology 130(4). DOI: 10.1016/j.jaci.2012.06.016.

Meskhi N. 2017. "Monitoring and control strategy of Brown marmorated stink bug (Halyomorpha halys) in Georgia." National Food Agency Report, 07.09.2017, Tbilisi, Georgia.

Mikaia N. 2018. "EPNs Steinernema carpocapsae and Heterorhabditis bacteriophora for Control of the Brown Marmorated Stink Bug (BMSB) Halyomorpha halys (Hemiptera, Pentatomidae)." Bulletin of the Georgian National Academy of Sciences 12, no. 3.

Milnes JM, Wiman NG, Talamas EJ, Brunner JF, Hoelmer KA, Buffington ML, Beers EH. 2016. "Discovery of an Exotic Egg Parasitoid of the Brown Marmorated Stink Bug, Halyomorpha halys (Stål) in the Pacific Northwest." Proceedings of the Entomological Society of Washington 118(3):466–470. DOI: 10.4289/0013-8797.118.3.466.

Milonas PG, Partsinevelos PK. 2014. "First report of brown marmorated stink bug Halyomorpha halys Stål (Hemiptera: Pentatomidae) in Greece." Bull OEPP/EPPO 44:183–186. DOI: 10.1111/epp.12129.

Ministry of Environmental Protection and Agriculture of Georgia. "News | Ministry of Environmental Protection and Agriculture of Georgia." (n.d.) Retrieved June 3, 2022, from https://mepa.gov.ge/En/News/Details/11703/.

Ministry of Finance of Georgia. 2017. "Information on the consideration of the conclusions and recommendations of the committees of the Parliament of Georgia on the 2018 draft state budget of Georgia" (1–89). Tbilisi, Georgia. Retrieved November 23, 2023, from https://www.mof.ge/images/File/biujetis-kanoni2018/II-wardgena/matrica.pdf.

Mirotadze M (2011). Determination of the optimal regime of drying of some nuts (hazelnuts) industrial species (in Georgian). Dissertation.

Mityushev I.M. (2016). First case of detection of the Marmorated stink bug in Russia. Zashchita i karantin rasteniy, No. 3, 48 (In Russian). On the approval of the unified list of quarantine items of the Eurasian Economic Union. 2016. URL: https://docs.eaeunion.org/docs/en-us/01213201/ cncd_06032017_158 (accessed: 28.01.2018). (In Russian)

Moore A., (2014). Brown marmorated stink bug Halyomorpha halys (Stal 1855) (Hemiptera: Pentatomidae). *Guam New Invasive Species Alert*, 2014, (1), 1–2.

Moris, J. (1991). Extension alternatives in tropical Africa. London: Overseas Development Institute.

Morrison WR III, Lee DH, Short BD, Khrimian A, Leskey TC (2016a). Establishing the behavioral basis for an attract-and-kill strategy to manage the invasive Halyomorpha halys in apple orchards. *J Pest Sci* 89:81–96.

Morrison WR III, Mathews CR, Leskey TC (2016b). Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. *Biol Control* 97:120–130.

Moura JC, Bonine CA, Viana JD, Dornelas MC, Mazzafera P (2010). Abiotic and Biotic Stresses and Changes in the Lignin Content and Composition in Plants. Journal of Integrative Plant Biology. 52(4), 360-376. https://doi.org/10.1111/j.1744-7909

Moura-Sobczak J, Souza U, Mazzafera P (2011). Drought stress and changes in the lignin content and composition in Eucalyptus. BMC Proceedings, 5(Suppl 7). https://doi.org/10.1186/1753-6561-5-s7-p103

Murvanidze M., G. Krawczyk, N. Inasaridze, L. Dekanoidze, N. Samsonadze, M. Macharashvili, S. Khutsishvili, S. Shengelaia (2018). Preliminary data on the biology of brown marmorated stink bug *Halyomorpha halys* (Hemiptera, Pentatomidae) in Georgia. Turk J Zool 42: 617-624.

Musolin D.M. (2017). Heteroptera: Pentatomoidea: variety of seasonal adaptations, control mechanisms seasonal development and climate change responses. Thesis for a degree Doctors of Biological Science. St. Petersburg. pp. 435 (in: Russian).

Nahar, N., & Pryor, S. W. (2013). Enzymatic hydrolysis and fermentation of crushed whole sugar beets. Biomass and Bioenergy, 59, 512–519. https://doi.org/10.1016/j.biombioe.2013.10.012

National Environmental Agency of Georgia. https://nea.gov.ge. Accessed 21 February 2021.

National Food Agency, Ministry of Agriculture of Georgia report presented on 20 November 2016. http://www.moa.gov.ge/

National Statistics Office of Georgia. Agriculture - Production of Permanent Crops. (2022, June 3). Retrieved June 3, 2022, from https://www.geostat.ge/en/modules/categories/196/agriculture

Nebieridze, E.L., Savenko, R., Kalandadze, L.P. 1957. Checklist of the Insects distributed in Georgia. Sakhelgami, Tbilisi, 296 pp.

Neimorovets, V.V. (2018). "Brown marmorated stink bug Halyomorpha halys (Heteroptera: Pentatomidae): morphology, biology, distribution and threats to agriculture in the Russian Federation (Analytical review)." *Plant Protection News*, 1(95), p. 11–16.

Nielsen, A.L., Shearer, P.W., Hamilton, G.C. 2008. "Toxicity of insecticides to Halyomorpha halys (Hemiptera: Pentatomidae) using glass-vial bioassays." *J. Econ. Entomol.*, 101:1439–1442.

Nielsen, A.L., Hamilton, G.C., Matadha, D. 2008. "Developmental rate estimation and life table analysis for Halyomorpha halys (Hemiptera: Pentatomidae)." *Environ. Entomol.*, 37:348–355.

Nikam, K.M., More, S.V. (2016). "Diversity of insects from Jangamhatti area, Chandgad, Kolhapur district of Maharashtra." *Biolife*, 4(1):209–212.

Noyes, J. (2015). Universal Chalcidoidea Database. http://www.nhm.ac.uk/research-curation/projects/chalcidoids/

Oda, M., Sugiura, T., Nakanishi, Y., Uesumi, Y. 1980. "Ecological studies of stink bugs attacking fruit trees. Report 1: the prevalence of seasonal observations by light trap, and the ecology on the occurrence of fruit trees and mulberry under field observations." *Bull. Nara Agric. Exp. Stn.*, 11:53–62.

Ohira, Y. 2003. "Outbreak of the stink bugs attacking fruit trees in 2002." *Plant Prot.*, 57:164–168.

Okuda, M.S., Yeargan, K.V. (1988). "Habitat partitioning by Telenomus podisi and Trissolcus euschisti (Hymenoptera: Scelionidae) between herbaceous and woody host plants." *Environ Entomol*, 17:795–798.

Osakabe, K., Tsao, C.C., Li, L., Popko, J.L., Umezawa, T., Carraway, D.T., Chiang, V.L. (1999). "Coniferyl aldehyde 5-hydroxylation and methylation direct syringyl lignin biosynthesis in angiosperms." *Proceedings of the National Academy of Sciences,* 96(16), 8955-8960. https://doi.org/10.1073/pnas.96.16.8955

Parker, B.L., Skinner, M., Gouli, S., Gouli, V., & Kim, J. 2015. "Virulence of BotaniGard® to Second Instar Brown Marmorated Stink Bug, Halyomorpha halys (Stål) (Heteroptera: Pentatomidae)." *Insects*, 6(2), 319–324. https://doi.org/10.3390/insects6020319

Patel, S., Singh, R. 2016. "Updated Checklist and Distribution of Mantidae (Mantodea: Insecta) of the World." *Int J Res Stud Zool*, 2(4). https://doi.org/10.20431/2454-941x.0204003

Peiffer, M., Felton, G. 2014. "Insights into the Saliva of the Brown Marmorated Stink Bug Halyomorpha halys (Hemiptera: Pentatomidae)." *PLoS ONE*, 9(2), e88483. https://doi.org/10.1371/journal.pone.0088483

Penca, C., Hodges, A. 2018. "First report of brown marmorated stink bug (Hemiptera: Pentatomidae) reproduction and localized establishment in Florida." *Florida Entomologist,* 101(4), 4p.

Petrisor, C., Stoian, G. 2017. "The role of hydrolytic enzymes produced by entomopathogenic fungi in pathogenesis of insects minireview." *Roman. J. Plant Prot.*, 10, 66–72.

Pfeiffer, D.G. March 30, 2009. "Brown Marmorated Stink Bug." Fact Sheet. Virginia Tech Department of Entomology. *Retrieved February* 24, 2011.

Pignede, G., Wang, H., Fudalei, F., Gaillardia, C., Semon, M., Nicaud, J.M. 2000. "Characterisation of an extra lipase encoded by L1P2 in Yarrowia lipolytica." *J Bacteriol.*, 182, 2802–2810.

Pollack, G. 2000. "Who, what, where? Recognition and localization of acoustic signals by insects." *Curr Opin Neurobiol.*, 10, 763–767. https://doi.org/10.1016/s0959-4388(00)00161-6

Pollack, G. 2000. "Who, what, where? Recognition and localization of acoustic signals by insects." *Curr Opin Neurobiol*, 10, 763–767.

Prete, F.R. 1999. "Prey recognition." In: Prete, F.R., Wells, H., Wells, P.H., Hurd, L.E. (ed). The praying mantids, *Johns Hopkins University Press*, Baltimore, Maryland, U.S.A., pp. 141–179.

Prete, F.R., Hurd, L.E., Branstrator, D., Johnson, A. 2002. "Responses to computer-generated visual stimuli by the male praying mantid, Sphodromantid lineola (Burmeister)." *Anim Behav*, 63, 503–510. https://doi.org/10.1006/anbe.2001.1955

Pushkar, T., Kavurka, V.V. 2016. "New data about the distribution of Hierodula transcaucasica in Ukraine." In: Problems of Modern Entomology, Uzhgorod, 15–17 Sept 2016, *Abstracts:* pp. 77–78. (in Russian)

Quarles, William. n.d. "IPM for the Brown Marmorated Stink Bug: Part 3." *Retrieved from* http://www.stopbmsb.org/

Rabitsch, W., Friebe, G.J. 2015. "From the west and from the east? First records of Halyomorpha halys (Stål, 1855) (Hemiptera: Heteroptera: Pentatomidae) in Vorarlberg and Vienna, Austria*." Beiträge zur Entomofaunistik*, 16, 115–139.

Rahman, M.M., Lim, U.T. 2017. "Evaluation of mature soybean pods as a food source for two pod-sucking bugs, Riptortus pedestris (Hemiptera: Alydidae) and Halyomorpha halys (Hemiptera: Pentatomidae)." PloS One, 12(4). https://doi:10.1371/*journal.pone.*0176187

Rehner, S.A., Minnis, A.M., Sung, H., Luangsa-ard, J.J., Devotto, L., Humber, R.A. 2011. "Phylogeny and systematics of the anamorphic, entomopathogenic genus Beauveria." *Mycologia*, 103, 1055–1073

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T.D., Hamilton, G., Haye, T., Herbert, A., Hoelmer, K.A., Hooks, C.R., Jones, A., Krawczyk, G., Kuhar, T., Martinson, H., Mitchell, W., Nielsen, A.L., Pfeiffer, D.G., Raupp, M., Rodrigues-Saona, C., Shearer, P., Shrewsbury, P., Venugopal, P.D., Whalen, J., Wiman, N.G., Leskey, T.C., Tooker, J.F. 2014. "Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae)." *J Integr Pest Manag*, 5:A1–A13.

Rice, Kevin B., Chris J. Bergh, Erik J. Bergmann, Dave J. Biddinger, Christine Dieckhoff, Galen Dively, Hannah Fraser, Tara Gariepy, George Hamilton, Tim Haye, Ames Herbert, Kim Hoelmer, Cerruti R. Hooks, Ashley Jones, Greg Krawczyk, Thomas Kuhar, Holly Martinson, William Mitchell, Anne L. Nielsen, Doug G. Pfeiffer, Michael J. Raupp, Cesar Rodriguez-Saona, Peter Shearer, Paula Shrewsbury, P. Dilip Venugopal, Joanne Whalen, Nik G. Wiman, Tracy C. Leskey, and John F. Tooker. 2014. "Biology, Ecology, and Management of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)." *J. Integ. Pest Mngmt.*, 5(3): 2014; DOI: http://dx.doi.org/10.1603/IPM14002

Rider, D.A., Zheng, L.Y., & Kerzhner, I.M. 2002. "Checklist and nomenclatural notes on the Chinese Pentatomidae (Heteroptera). II. Pentatominae." *ZOOSYST. ROSSICA*, Vol. 11, 135-153.

Rijal, J., Sudan, G. 2018. "Characterizing Brown Marmorated Stink Bug Injury in Almond, a New Host Crop in California." *Insects*, 9(4), 126. https://doi.org/10.3390/insects9040126

Rijal, Jhalendra, and Sudan, Gyawaly. 2018. "Characterizing Brown Marmorated Stink Bug Injury in Almond, a New Host Crop in California." *Insects*. DOI: 10.3390/insects9040126

Rilling, S., Mittelsteadt, H., Roeder, K.D. 1959. "Prey recognition in the praying mantid." *Behaviour*, 14, 164–184. https://doi.org/10.1163/156853959X00063

Roberts, D.W., Hajek, A.E. 1992. "Entomopathogenic fungi as bioinsecticides." In: G.F. Leatham (ed). *Frontiers in industrial mycology*. New York: Chapman & Hall, pp. 144–159.

Romanowski, J., Battiston, R., Hristov, G. 2019. "First Records of Hierodula transcaucasica Brunner von Wattenwyl, 1878 (Mantodea Mantidae) in the Balkan Peninsula." *Acta Zoologica Bulgarica*, 71(2), 297–300.

Saito, Y., Saito, S., Ohmori, Y., and Yamada, K. 1964. "Studies on bionomics of the bean bugs occurring in mountain areas, with particular reference to that of Halyomorpha picus and to the insecticidal tests in laboratory and field." Jpn. *J. Sanit. Zool.*, 15, 7Ð16.

Sambrook, J., Russell, D.W. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold *Spring Laboratory Press*, Cold Spring Harbor.

Šapina, I., Šerić Jelaska, L. 2018. "First report of invasive brown marmorated stink bug Halyomorpha halys (Stål, 1855) in Croatia." *EPPO Bull*, 48(1), 138–143.

Saruhan, I., Tuncer, C. 2010. "Research on damage rate and type of green shieldbug (Palomena prasina L. Heteroptera: Pentatomidae) on hazelnut." *Anadolu Tarım Bilimleri Dergisi*, 25, 75–83.

Sathe, T. V.; Prakash Bhoje. July 2000. Biological Pest Control. Edition: 1st. *Daya Publishing House*, New Delhi. ISBN: 10: 8170352258, ISBN 13: 9788170352259.

Sauer, C. 2012. "Die Marmorierte Baumwanze tritt neu im Deutschschweizer Gemüsebau auf." Extension Gemüsebau, Forschungsanstalt Agroscope Changins-Wädenswil, Gemüsebau Info 28(12), 4–5.

Šeat, J. 2015. "Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) a new invasive species in Serbia." *Acta Entomol Serb*, 20, 167–171.

Sichinava, N. 2005. "Biological and economic characteristics of hazelnut spread in Samegrelo and scientific basis of its production." (in Georgian). Tbilisi, 64.

Simov, N. 2016. "The invasive brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) already in Bulgaria." Ecol Mont, 9, 51–53.

Spec-Kniga.ru. (n.d.). "Methods for accounting of harmful and beneficial insects." Библиотека специализированной литературы. Retrieved September 20, 2022, from https://www.spec-kniga.ru/rastenievodstvo/poleznye-nasekomye-sada-i-ogoroda/poleznye-nasekomye-sada-i-ogoroda-metody-ucheta-vrednyh-i-poleznyhnasekomyh.html?fbclid=IwAR3rQJZfl93eaAtKQ4mCl6GYjH7mhLdqIUH1k2mNpgtMGJ4XVEVT9Tt5so.

Stahl, Judith; Tortorici, Francesco; Pontini, Marianna; Bon, Marie-Claude; Hoelmer, Kim; Marazzi, Cristina; Tavella, Luciana; Haye, Tim. 2018. "First discovery of adventive populations of Trissolcus japonicus in Europe." Journal of Pest Science, 92(2), 371–379. doi:10.1007/s10340-018-1061-2.

Starner, K., White, J., Superlock, F., Kelley, K. 2008. "Assessment of pyrethroid contamination of streams in high-use agricultural regions of California." ACS Symposium Series. DOI: 10.1021/bk-2008-0991.ch004.

Stock, S. P., & Goodrich-Blair, H. 2012. "Nematode parasites, pathogens and associates of insects and invertebrates of economic importance." In: Manual of Techniques in Invertebrate Pathology, 373–426. https://doi.org/10.1016/B978-0-12-386899-2.00012-9.

Szűcs, Marianna; Gut, Larry; Wilson, Julianna; Pote, John. July 24, 2019. "Biological control of brown marmorated stink bug in Michigan." Michigan State University, Department of Entomology. Retrieved December 3, 2019.

Tada, N., M. Yoshida, and Y. Sato. 2001. "Monitoring of forecasting for stink bugs in apple 2. The possibility of forecasting with aggregation pheromone." Annu. Rep. Plant Prot. North. Jpn., 52, 227–229.

Takahashi, S. 1930. "Halyomorpha picus Fab." In Insect Pests on Fruit Trees, pp. 617–620.

Talamas, E.; Buffington, M.; Hoelmer, K. 2013. "New synonymy of Trissolcus halyomorphae Yang." Journal of Hymenoptera Research, 33, 113–117. https://doi.org/10.3897/jhr.33.5627.

Talamas, E.J., Herlihy, M. V.; Dieckhoff, C.; Hoelmer, K. A.; Buffington, M.; Bon, M.-C.; Weber, D. C. 2015. "Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae) emerges in North America." Journal of Hymenoptera Research, 43, 119–128. doi:10.3897/jhr.43.4661.

Tarasco, E., Clausi, M., Rappazzo, G., Panzavolta, T., Curto, G., Sorino, R., Oreste, M., Longo, A., Leone, D., & Tiberi, R. 2015. "Biodiversity of entomopathogenic nematodes in Italy." Journal of Helminthology, 89(3), 359–366. https://doi.org/10.1017/S0022149X14000194.

Tavella, L., Arzone, A., Sargiotto, C., & Sonnati, C. 1997. "Coreidae and Pentatomidae harmful to hazelnuts in northern Italy (Rhynchota: Heteroptera)." Acta Hort., 445, 503–509.

Tavella, L., Arzone, A., Miaja, M. L., & Sonnati, C. 2001. "Influence of bug (Heteroptera, Coreidae and Pentatomidae) feeding activity on hazelnut in northwestern Italy." Acta Hort., 556(556), 461–468. https://doi.org/10.17660/ActaHortic.2001.556.68.

Tishenko, B. P. 1986. Insect Physiology, p. 303. (in Russian).

Tobin, P.C.; Nagarkatti, S.; Loeb, G.; Saunders, M.C. 2008. "Historical and projected interactions between climate change and insect voltinism in a multivoltine species." Glob. Change Biol., 14, 951–957. https://doi.org/10.1111/j.1365-2486.2008.01561.x.

Toju, H., Tanabe, A.S., Yamamoto, S., & Sato, H. 2012. "High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples." PLoS One, 7(7), e40863. doi:10.1371/journal.pone.0040863.Tsintsadze Nina, (23 Mar 2018) https://www.ebrd.com/home

Tuncer, C., Saruhan, İ., & Akça, İ. 2014. "Seasonal occurrence and species composition of true bugs in hazelnut orchards." Acta Hortic, 1052:263–268.

Tuncer, C., Saruhan, I., & Akca, I. 2005. "The insect pest problem affecting hazelnut kernel quality in Turkiye." In Tous, J., Rovira, M., Romera, A. (Eds.), Proceedings of the VI International Congress on Hazelnut, June 14, 2004, Tarragona-Reus, Spain, 686: 367–376.

Ullah, S., Li, Z., Zuberi, A., Arifeen, M. Z., & Baig, M. M. 2019. "Biomarkers of pyrethroid toxicity in fish." Environmental Chemistry Letters, 1-29. doi:10.1007/s10311-018-

Vellinga, E. C. 1988. "Glossary." In C. Bas, Th.W. Kuyper, M.E. Noordeloos, E.C. Vellinga (Eds.), Flora Agaricina Neerlandica, Critical monographs on families of agarics and boleti occurring in the Netherlands, vol 1. A.A. Balkema, Rotterdam, pp. 54–64.

Venugopal, P. D., Coffey, P. L., Dively, G. P., & Lamp, W. O. 2014. "Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges." PLoS ONE, 9(10). doi:10.1371/journal.pone.0109917

Vétek, G., Papp, V., Haltrich, A., & Rédei, D. A. 2014. "First record of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Heteroptera: Pentatomidae), in Hungary, with description of the genitalia of both sexes." Zootaxa, 3780(1):194–200. doi:10.11646/zootaxa.3780.1.8

Vyas, P. R., & Deshpande, M. V. 1989. "Chitinase production by Myrotheciumverrucaria and its significance for fungal mycelia degradation." J Gen Appl Microbiol., 35:343–350.

Walgenbach, J. (Ed.). 2017. "Where is BMSB?" Accessed online from: http://www.stopbmsb.org/ (last update 20 September 2017).

Walker, K. 2011. "Brown marmorated stink bug (Halyomorpha halys)." AM. Available online: PaDIL - http://www.padil.gov.au. (last update 25 November 2011)

Walker, S. E., & Lorsch, J. 2013. "RNA Purification – Precipitation Methods." Methods in Enzymology, 337–343. doi:10.1016/b978-0-12-420037-1.00019-1

Watanabe, K. 1996. "Characteristic of damages of Lygocoris (Apolygus) lucorum (Meyer-Dur) (Heteroptera: Miridae) and Halyomorpha halys (Stål) (Heteroptera: Pentatomidae) on cherry." Annu. Rep. Plant Prot. North. Jpn., 47: 143–144

White, T. J., Bruns, T. D., Lee, S., & Taylor, J. 1990. "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics." In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR Protocols. Academic Press, San Diego, CA, USA, pp. 315–322.

Will, T., Steckbauer, K., Hardt, M., & Bel, A. J. 2012. "Aphid Gel Saliva: Sheath Structure, Protein Composition and Secretory Dependence on Stylet-Tip Milieu." PLoS ONE, 7(10). https://doi:10.1371/journal.pone.0046903

Wiman, N. G., Walton, V. M., Shearer, P. W., Rondon, S. I., & Lee, J. C. 2014. "Factors affecting flight capacity of brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae)." J. Pest Sci., 88:37–47

Wyniger, D., & Kment, P. 2010. "Key for the separation of Halyomorpha halys (Stål) from similar-appearing pentatomids (Insecta: Heteroptera: Pentatomidae) occurring in Central Europe, with new Swiss records." Mitt Schweiz Entomol Ges, 83:261–270.

Yanagi, T., & Hagihara, Y. 1980. "Ecology of the brown marmorated stink bug." Plant Prot., 34: 315–321.

Yang, Y., Wu, N., & Wang, C. 2018. "Toxicity of the pyrethroid bifenthrin insecticide." Environmental Chemistry Letters.

Yang, Z-Q., Yao, Y-X., Qiu, L-F., & Li, Z-X. 2009. "A new species of Trissolcus (Hymenoptera: Scelionidae) parasitizing eggs of Halyomorpha halys (Heteroptera: Pentatomidae) in China with comments on its biology." Annals of the Entomological Society of America, 102(1):39-47.

Zaitseva, I. 1998. "Synopsis on the fauna of heteropterans of Georgia." Georgian Academy of Science, 2, 76.

Zhang, A., Khrimian, A., Aldrich, J. R., Leskey, T. C., & Weber, D. C. 2013. "Compositions and methods to attract brown marmorated stink bug (BMSB), Halyomorpha halys. U.S. Provisional."

Zhimerikin, V. N., & Guliy, V. V. 2014. "Brown marmorated stink bug." Protection and quarantine of plants, 4, 40-43. (In Russian)

Zhu, G., Bu, W., Gao, Y., & Liu, G. 2012. "Potential geographic distribution of brown marmorated stink bug invasion (Halyomorpha halys)." PLOS One, 7(2), e31246.