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Original Research Article

Analysis of Insect's Substances Constituent Compounds of Ground beetles (*Pterostichus melanarius*) and Evaluation of Its Antifungal Activity

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Abstract: *Pterostichus melanarius* is a member of the family Carabidae belonging to Tribe Pterostichini. No existing character that supports the idea based on the high diversity within the tribe, the fact that the tribe is a clade. The aim of the research was to examine the antifungal activity and the chemical composition of insect parts ground beetle (*Pterostichus melanarius*). We killed the ground beetle insects using a killing jar then allowed them to air dry, ground them, and store at room temperature. Following a three-day maceration process of the 20 g of powdered material at periodic intervals with agitation under decreased pressure at 40oC the extract was condensed. The outcome was a whitish product of ethyl acetate and a brownish product of ethanol. The dishes were allowed to harden after pouring the SDA medium on them. Then, each fungus was stripped of a 5 mL disc (using sterile cork borer) and placed on top of the culture medium. The petri dishes will be stored at 25o C +/-2 at a constant temperature over a period of 7 days. The antifungal activity of the extracts can be determined by using a ruler to measure the diameter of the inhibitory zone in millimetres (mm). There are eleven major compounds, namely: 2-Tetradecene, Benzenepropanoic acid, 4-methyl, n-Tetradecanoic acid, hexadecanoic acid, ethyl ester, 1,10-Cycloeicosanedione, hexacosanedioic acid, naphthalene-1,4-diol, cis-1,2-Cyclohexanediol, 15-Nonacosanone and 4-(hydroxymethyl) benzaldehyde. *Aspergillus flavus* activity against metabolite was quite high in ground beetles (*Pterostichus melanarius*) (26.07±0.48).

Keywords: *Pterostichus Melanarius*, Ground Beetles, Compounds, Antifungal Activity.

INTRODUCTION

Hygrophilic and eurythermic are used to describe *Pterostichus melanarius*. This species is prohibited in all except pure sand and gravel, and otherwise has a wide distribution in all soils [1, 2]. Urban areas, roadsides, woodlands, meadows, grasslands, lake and river banks and arable land are only some of the numerous places you may find *P. melanarius* due to its generalist feeding behaviour and the fact that it is able to tolerate a wide range of soil types and temperatures. *Pterostichus melanarius* are also able to spread their range very fast with an average daily dispersal distance ranging between 2.5-5 m. On the other hand, people can go up to 44 meters in a single night. The daily displacement distance may increase as the process of finding a mate in the late summer proceeds. This rapid extension of its range might be enabled by wing dimorphism, and flight capabilities, although *Pterostichus melanarius* can walk quite long distances. A preliminary investigation suggested that it is brachyptery, not macroptery, that is the dominant wing shape in *Pterostichus melanarius*, and that wing dimorphism is inherited through simple mendelian genetics. Whereas the macropterous insects possess long hindwings and are able to fly, the brachypterous insects have short hindwings and cannot fly. Brachypterous individuals increase in number and macropterous individuals comprise a higher proportion of the newly formed populations [3]. The fact that one can fly is one advantage because there may be a reduction in the intraspecific rivalry of resources and ease of expansion of the range of the individual in the newly found areas. In Europe, where it is not common, macropetry comprises less than 2% of *Pterostichus melanarius* populations. Insects are important agents in biological management (biocontrol)

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when it comes to both managed and unmanaged ecosystems. Natural control services are often provided on agricultural fields because biocontrol agents (also referred to as natural enemies), such as insect predators and parasitoids, are present there [4, 5]. The agroecosystems have the advantage of the natural control services since they keep the pesticide inputs at the minimum level possible, which contributes to maintaining the equilibrium of the ecosystems and reducing the effects of pesticides resistance. To distinguish and promote the beneficial functions of predators in ecologies, insect predators fundamentally interact with the organisms that they prey on. The first step is to find out how insect predators perceive their prey and how they can decide whether they are worth eating or not. Having this information, we are able to make more accurate prediction on the capabilities of predators as field-applied biocontrol agents. This data is of particular significance when studying predatory species as it is especially important to have this data when studying omnivorous predatory species [6-8]. In this regard, it is paramount to cast some light on the sensory processes of prey detection and discrimination with a view of understanding the reasons as to why certain species are more susceptible to increased predation risks especially in situations where the predator has access to other species that offers them alternative food sources [9-11]. To better manage agroecosystems for increased diversity and abundance of insect predators, which improves their ecological functioning, it is essential to understand the fundamental ecological characteristics of predatory insects' eating patterns. The objective of the study was to examine antifungal activity and chemical composition of insect parts (ground beetle (*Pterostichus melanarius*) insect parts).

MATERIALS AND METHODS

Selecting and Organizing

We set up a pitfall trap half full of 100% propylene glycol at each sampling location to trap carabids so as to test the response hypothesis. After a 14 days period of sampling, the pitfalls in each field location were collected. This sample was collected and reset on a daily basis during these samples, then again 14 days later. The sample was taken thrice consecutively in each of the ten barley fields. Among the ten canola sites, seven were with four sampling periods and three with single sampling periods. The contents of each pitfall were collected and filtered in 95% ethanol after the propylene glycol was filtered out. All samples were checked on carabids. The most prevalent ground beetle in this system, *Pterostichus melanarius*, was identified. Since of their larger size and easy manipulation, individuals of Pterostichus melanarius were measured using digital callipers the length of the elytra, a plausible proxy evaluation of the size of ground beetles. The large amount of *Pterostichus melanarius* individuals did not justify the extra resources beyond storing in EtOH. As we only wanted to measure the functional attribute of the individuals, we did not assign any species to the other pinned carabids. All carabid samples are located in the Invertebrate Collection at the University of Calgary. We applied a lab experiment to test the second hypothesis which is the effect hypothesis using *Pterostichus melanarius* collected at the dry pitfalls and opportunistic sites. The beetles which fed on these insects were of different sizes, and we had a view of how many of them were eaten. To ensure a smooth trial, the beetles were stored at room temperature and given a constant supply of water and food, in this case, canola seeds and cooked cat food.

Extract Preparation

We put the ground beetle insects in a killing jar and allowed them to air dry, which was followed by grinding them up, and keeping them at room temperature. The extract was then concentrated under reduced pressure at 40oC, after three days of subjecting the 20 g of the powdered material to a maceration process. Ethyl acetate produced a whitish residue and ethanol produced a brownish residue. Ethyl acetate and ethanol extracts were dissolved in 8 mL of dimethyl sulfoxide (DMSO) to make a stock solution with a concentration of 10 mg/mL.

The Study of the Antifungal Activity of Extracts of *Pterostichus Melanarius*

The antifungal activity of ethyl acetate and ethanol extracts has been investigated by using a combination of ethol with sabouraud dextrose agar (SDA). Then, 0.1 mL was pipette transferred to a Petry dish of each concentration. The dishes were allowed to dry after pouring the SDA medium on top. Then, a 5 mL disc was taken off each fungus using a sterile cork borer and placed on top of the culture medium. The petri dishes are incubated at a temperature of 25oC +/-2. To determine the antifungal effect of the extracts, the diameter of the inhibitory zone measured in millimetres (mm) using a ruler is used as an indicator of the antifungal activity of the extracts [12, 13]. Determination of the percentage inhibition of diameter growth (PIDG) The following equation was used to calculate the values of percentage inhibition of diameter growth (PIDG):

The percentage of diameter growth inhibition (PIDG) in % = Diameter of sample – Diameter of control / diameter of the control × 100

Statistical Analysis

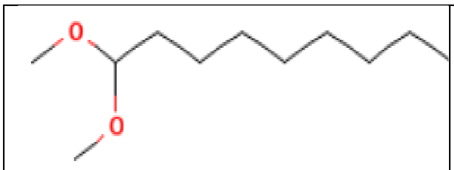
An ordered categorical generalised linear model was used to analyse the data for the effect hypothesis. As the response variable, the prey size was divided into four ordered categories: the smallest, medium, and largest. This analysis was done using the program. We first examined a simple model where the linear relationship between the length of the elytra of Pterostichus melanarius and the other model was used to determine the prey category. The second model was based on the first one except that a categorical variable that represented the angle of attack between the beetle and its prey was added. To compare models based on AIC, we used DHARMa 0.4.6 in R 4.3.2 to assess the model fit. The null hypothesis was tested at a significance level of 0.05; that is, whether a model term had no effect on the size category of prey that was chosen.

RESULTS AND DISCUSSION

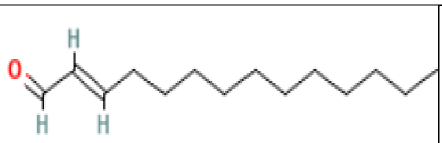
The GC-MS results of the Ground beetle ethanol extract are presented in Table 1. Eleven main chemicals that are dominant: Nonanal dimethyl acetal $C_{11}H_{24}O_2$, 2-Tetradecene, (2E) $C_{14}H_{26}O$, Benzenepropanoic acid, 4-methyl $C_{17}H_{15}F_3O_3$, n-Tetradecanoic acid $C_{19}H_{42}O_2Si_2$, Hexadecanoic acid, ethyl ester $C_{29}H_{53}NO_4$, 3-Hydroxy-octadecanoic Acid Ethyl Ester $C_{20}H_{41}NO_3$, 1,10-Cycloeicosanedione $C_{20}H_{36}O_2$, Hexacosanedioic acid $C_{26}H_{50}O_4$, Naphthalene-1,4-diol $C_{10}H_8O_2$, cis-1,2-Cyclohexanediol $C_6H_{12}O_2$, 15-Nonacosanone $C_{29}H_{58}O$ and 4-(hydroxymethyl)-benzaldehyde $C_8H_8O_2$. Gas chromatography is one of the most important tools in chemistry, but it has a very wide variety of applications, such as quantitative and qualitative analysis of mixtures, purification of compounds, determination of thermo-chemical constants, including activity coefficients, vapour pressure, and heats of solution. Cycloeicosane had an antimicrobial and anti-oxidant effect, and the presence of 1-tetradecene and 2-tetradecene in the methanol extract indicated that the extract contained anti-cancer compounds. These trace chemicals have a high range quality of 64 to 96 percent and are associated with severe swelling effect in cells, the appearance of sickle and tear drop cells and other effects. Most of the substances synthesized out of insects are organic and these compounds do not become any exception. The area and percentage of quality of bis -(2-ethylhexyl)phthalate was the lowest and n-hexadecanoic acid had the highest. The reason why n-hexadecanoic acid and 9-octadecenamide are similar might be in the number of carbon atoms, the sequence of these atoms and the functional groups present in their compounds.

Table 1: Ethanolic extract of Ground beetles (*Pterostichus melanarius*) GC-MS

No.	Compounds	Molecular Formula	Molecular Weight
1.	Nonanal dimethyl acetal	$C_{11}H_{24}O_2$	188.31 g/mol
2.	2-Tetradecene, (2E)	$C_{14}H_{26}O$	210.36 g/mol
3.	Benzenepropanoic acid, 4-methyl	$C_{17}H_{15}F_3O_3$	324.29 g/mol
4.	n-Tetradecanoic acid	$C_{19}H_{42}O_2Si_2$	358.7 g/mol
5.	Hexadecanoic acid, ethyl ester	$C_{29}H_{53}NO_4$	479.7 g/mol
6.	3-Hydroxy-octadecanoic Acid Ethyl Ester	$C_{20}H_{41}NO_3$	343.5 g/mol
7.	1,10-Cycloeicosanedione	$C_{20}H_{36}O_2$	308.5 g/mol
8.	4-(hydroxymethyl)benzaldehyde	$C_8H_8O_2$	136.15 g/mol
9.	Hexacosanedioic acid	$C_{26}H_{50}O_4$	426.7 g/mol
10.	Naphthalene-1,4-diol	$C_{10}H_8O_2$	160.17 g/mol
11.	cis-1,2-Cyclohexanediol	$C_6H_{12}O_2$	116.16 g/mol
12.	15-Nonacosanone	$C_{29}H_{58}O$	422.8 g/mol

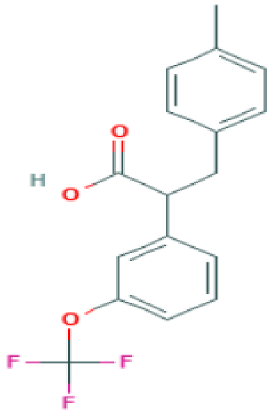


Nonanal dimethyl acetal

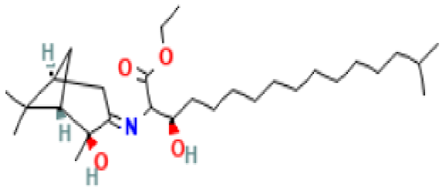


2-Tetradecene, (2E)

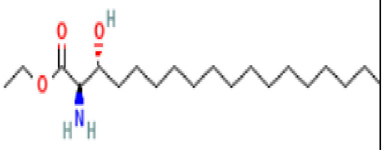
n-Tetradecanoic acid



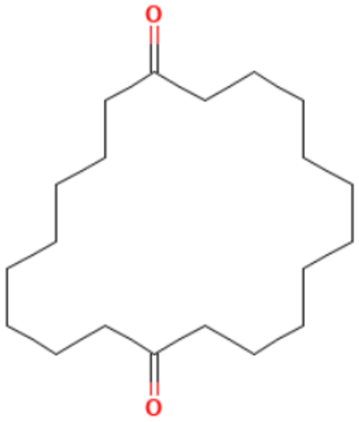
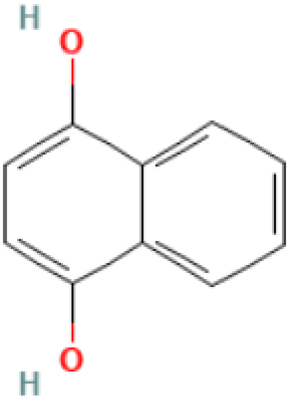
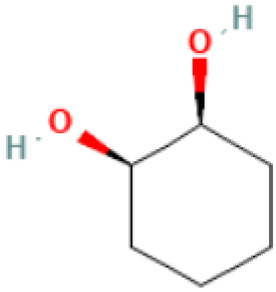
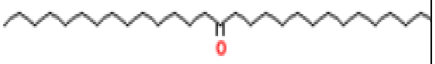
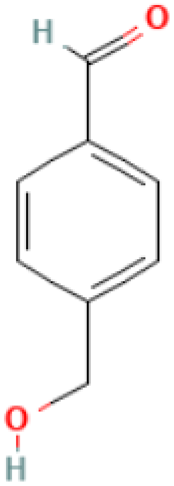
Benzenepropanoic acid, 4-methyl



Hexadecanoic acid, ethyl ester



3-Hydroxy-octadecanoic Acid Ethyl Ester

 **Hexacosanedioic acid**	 **1,10-Cycloeicosanedione** **15-Nonacosanone**	 **Naphthalene-1,4-diol**
 **cis-1,2-Cyclohexanediol**	 **15-Nonacosanone**	 **4-(hydroxymethyl)-benzaldehyde**

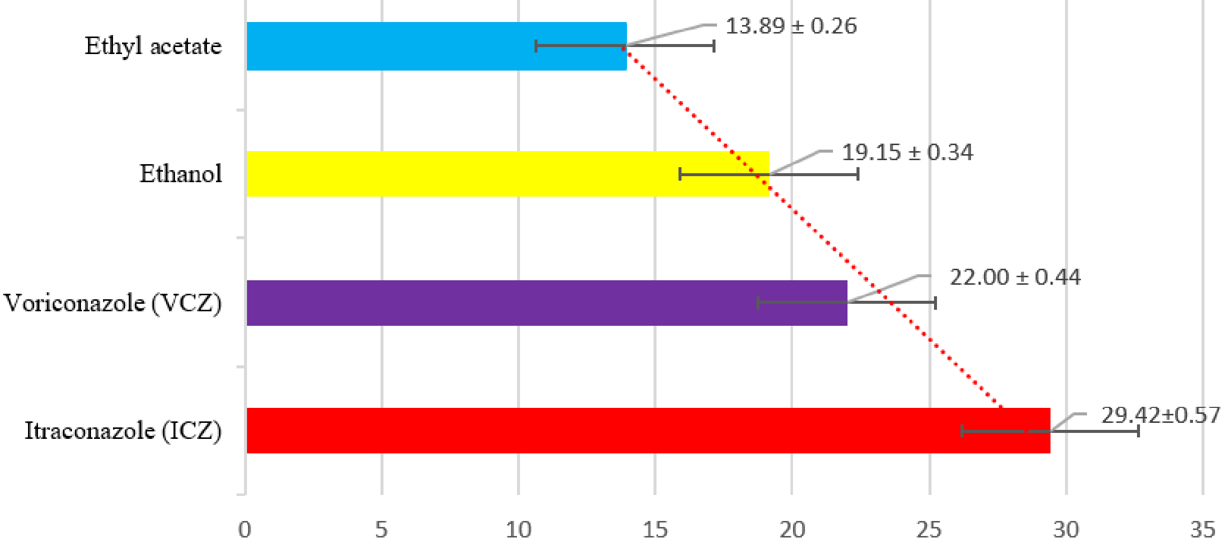


Figure 1. Antifungal activity of natural chemical compounds of Ground beetles (Pterostichus melanarius) against *Alternaria alternaria*.

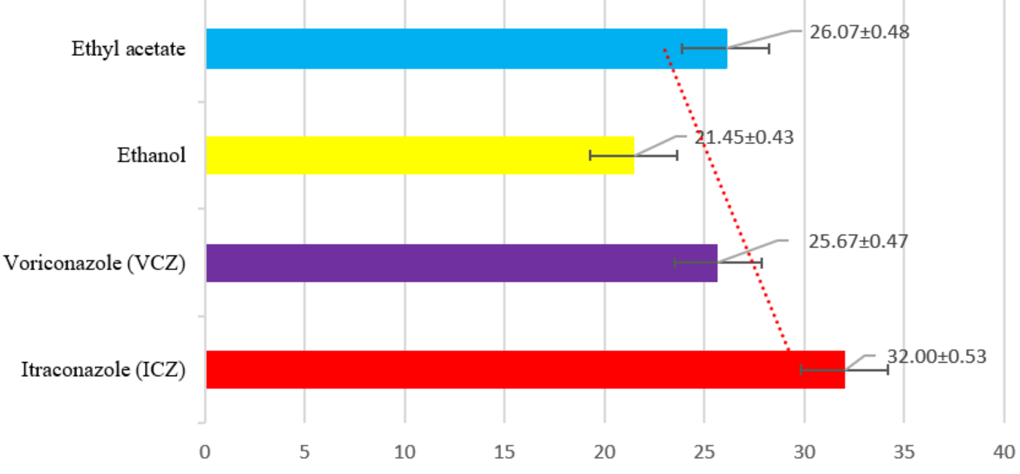


Figure 2. Antifungal activity of natural chemical compounds of Ground beetles (Pterostichus melanarius) against *Aspergillus flavus* .

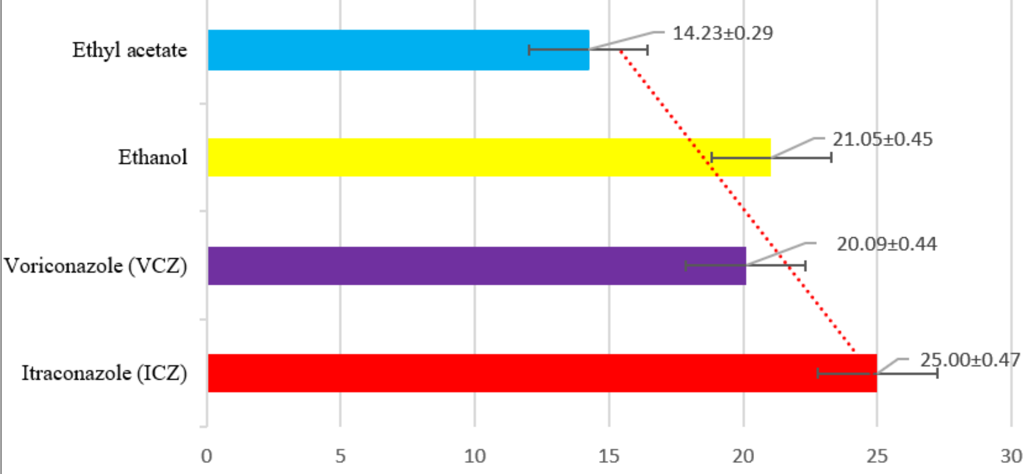


Figure 3. Antifungal activity of natural chemical compounds of Ground beetles (Pterostichus melanarius) against *Trichophyton rubrum.*

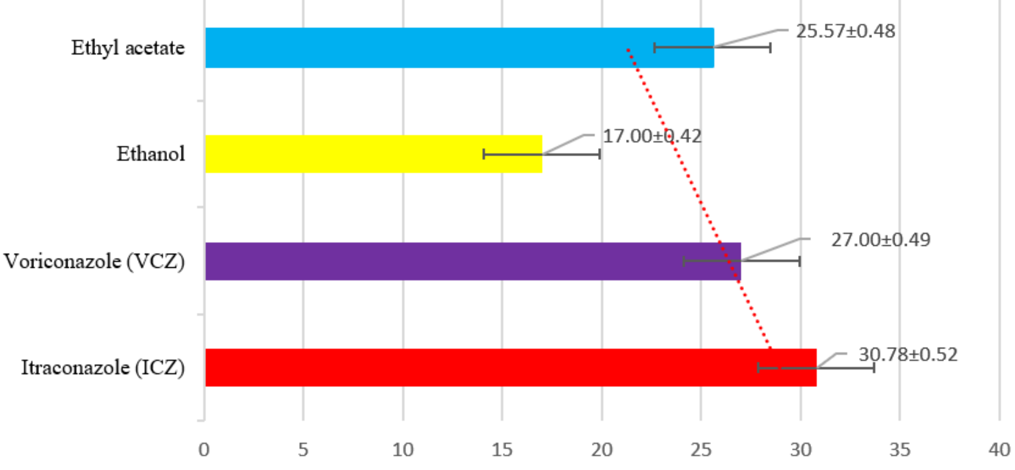


Figure 4. Antifungal activity of natural chemical compounds of Ground beetles (Pterostichus melanarius) against *Fusarium oxyporum.*

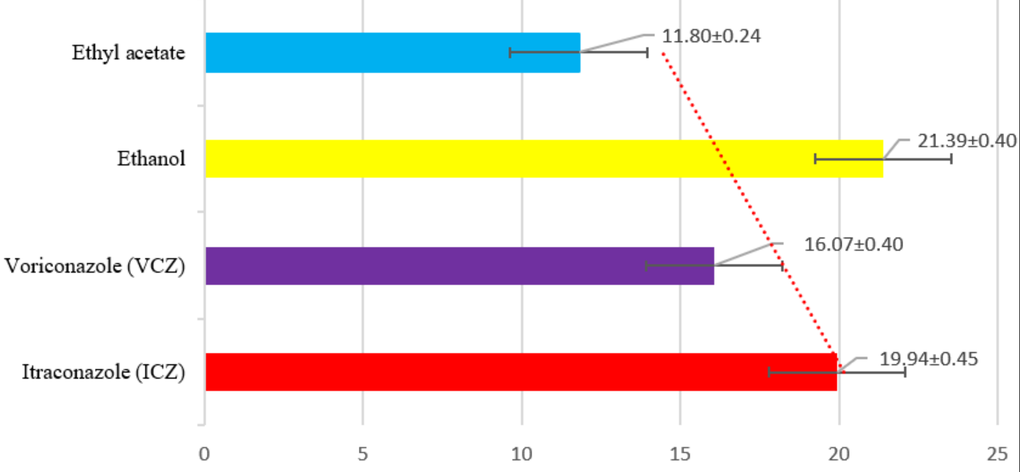


Figure 5. Antifungal activity of natural chemical compounds of Ground beetles (Pterostichus melanarius) against *Cladosporium herbarum*.

The antifungal effect of Ethyl acetate and Ethanol extract of natural chemical compounds of Ground beetles (*Pterostichus melanarius*) against fie fungi in comparison with standards (Voriconazole (VCZ) and Itraconazole (ICZ)) recorded: (13.89 ± 0.26, 19.15±0.34, 22.00±0.44 and 29.42±0.57 respectively for *Alternaria alternaria*), (26.07±0.48, 21.45±0.43, 25.67±0.47, 32.00±0.53 respectively for *Aspergillus flavus*), (14.23±0.29, 21.05±0.45, 20.09±0.44, 25.00±0.47 respectively for *Trichophyton rubrum*), (25.57±0.48, 17.00±0.42, 27.00±0.49, 30.78±0.52 respectively for *Fusarium oxyporum*), (11.80±0.24, 21.39±0.40, 16.07±0.40, 19.94±0.45 respectively for *Cladosporium herbarum*). Ground beetles (*Pterostichus melanarius*) metabolites was very highly active against *Aspergillus flavus* (26.07±0.48). Due to their epigaeic polyphagous predator status, ground beetles are considered to be one of the most significant predatory insect orders in the temperate areas. The carabid activity can be either diurnal or nocturnal depending on the species identification and its ecological characteristics. On average, they are able to consume almost the weight of their body in a day. Some of the agricultural pests preyed upon by many carabid species, include slugs, wireworms, aphids, dipteran eggs and midges, and lepidopteran caterpillars. The majority of carabid predators are omnivores, i.e., they feed on insects as well as a wide range of weed seeds once they have shed their own. Carabids are important to the agroecosystem because of their diverse food preferences and their aggressive predatory skills. Their diverse feeding behavior makes it challenging to predict the ability of omnivorous carabids to offer biocontrol service under agroecosystem context. Given the abundance of prey in arable fields, the evolution of seed eating habits is puzzling and mostly unexplored. That is why, in the real life, it is difficult to guess which species of seeds or prey organisms would be the most vulnerable to the increased carabid attacks [14]. Some have speculated that omnivorous carabids mix seed consumption with their prey eating habits because prey feeding alone isn't enough for survival and development, while others have suggested that omnivorous carabids only seek out seed consumption when prey species are scarce or inaccessible. It was originally assumed that seed feeding was of specific interest to some of the species of granivorous carabids. These animals obtain most of their foodstuff by a type of seed. Moreover, the seed-eating behaviours of omnivorous carabids are also opportunistic in nature in that they are mostly triggered by the unavailability or inaccessibility of other food sources. Due to the fact that seeds are part and parcel of the diets of a number of carabid taxa, seed feeding behaviours in carabids often transcend the artificial limits to which the dietary specialisation argument (omnivory vs. granivory) reduces them. These results suggest that the seed-eating behavior of carabid species may have evolved as an adaptation to unknown biological needs not necessarily restricted to the granivorous carabids. Consequently, research into the ecological effects of omnivorous carabids' seed-eating habits on carabids' ecological function in agroecosystems is crucial [15, 16]. Omnivorous carabids' seed-eating habits are probably motivated by actions that are meant to meet particular physiological demands. Traditional wisdom held that carabids' seed-feeding and seed-selection judgements were based only on chance encounters. Both laboratory and field studies have indicated, however, that carabid predators actively choose one or more of them given a choice of several seed species. To gratify themselves, carabids have to sort out seeds of different species, and to select the best one, they must first of all evaluate them according to their merits. Carabids rely on their many sensory systems to gather accurate information obtained from seeds in order to carry out their functions. Mechanisms of sensory and behavioural regulation of the carabid seed choice and discrimination remain poorly known. Although the readings of an olfactometer can be biased by the aromas of seeds, it remains unclear to which extent the chemoperception can drive accurate decisions towards seed selection. Very probably, both the visual and gustatory systems must be involved in the choice of the right seed species. Due to this uncertainty, it is difficult to tell what sensory cues carabid predators respond to in order to effectively search and locate suitable seed species. Research on the sensory ecology of omnivorous carabids' ability to detect and discriminate amongst seeds is crucial for understanding which chemical or physical characteristics of seeds make them more susceptible to attacks by carabids. This should improve our understanding of the contribution of carabid predators to the functioning of agroecosystems, and we may even be in a position to assemble the biological and environmental factors that led to the shift in seed-eating behaviour of omnivorous carabids. Seed detection and discrimination sensory ecology remains a mystery due to a lack of in-depth sensory investigations and the intricacy of carabid predators' eating patterns. Despite the high reliance of carabids on visual cues to inform their hunter behaviours, the strategies generally do not work when their prey objects are made immobile. Consequently, carabid visual sensors would prove to be of greater use in hunting highly mobile prey as they would seem to be more adapted to detecting prey movement. Since seeds tend to be cryptic and difficult to contrast with the soil, carabids cannot rely on visual sensors to detect sessile prey or weed seeds. The other theory is that carabids recognize and discriminate seed species and immobile prey using their chemoreceptors. Indeed, chemoperception is the major sensory modality in prey detection and selection in both specialised carnivores and unspecialised omnivores, according to mechanistic studies. To identify suitable or preferred prey species, the chemosensors detect volatile chemicals (i.e., prey odours) released by prey under the use of chemosensors on their palps and the antennae of the carabids. In the carabid literature, there is a deficiency of comparative mechanical data on seed discrimination and detection in the carabid literature. Thus, whether the seed selection of carabid seed predators is mainly determined by the perception of seed odours is an open question. Here we will explore the sensory ecology of the capacity of carabid seed predators to perceive and recognize seeds. The initial objective was to determine how carabids sense seeds and the sensory clues that they utilize to detect and differentiate between seed of various species. Omnivory is used in this study as the capacity of carnivorous species to feed on both plant and animal foods, without regard to their feeding specialisation or breadth of diet. Studies conducted with sensory manipulation protocols and multichoice seed feeding bioassays have demonstrated that carabids more or less depend on olfactory perception to detect and differentiate among the various species of seeds. The presence of volatile compounds known as long-chain hydrocarbons that were present in the epicuticular lipids on the seed coat was also an important factor in the choice of the right seed species. Hydrocarbons in seed surfaces that are isolated and identified seem to encode chemical information on the species-specific fatty acid composition of seeds. In that regard, it can be assumed that the information coded in hydrocarbons that are deposited on the surface of the seeds will be used by the carabid predators to know the fatty acid content in the species based on the coding information that was transmitted in the hydrocarbons deposited on the surface of the seeds to read it. Carabids can feed on a broad range of seed species containing lipids and this indicates that seed species with lipids are more susceptible to field predation. The reason is that seed physical characteristics define the costs of handling the seeds and these costs are not very diverse. Besides that, P. melanuary appears to be expanding its range towards the center of North America, which is not surprising since it is an invasive species, which was introduced in the coasts. Pterostichus melanarius is an important introduced natural enemy, but no one has ever tried to describe its life cycle, how it reacts to farming methods, or where it is likely to be found. This compilation of information about *Pterostichus melanarius* and its application in agricultural systems was inspired by our previous work on the biology and importance of the important exotic natural enemy Harmonia axyridis. The habitat generalist *Pterostichus melanarius* can be a component of the natural enemy community in crop fields in the eastern United States, and is predominantly a predator. This species can play a significant role as a predator in some cropping systems and a minor one in others. As an illustration, a series of field experiments conducted at the same research farm in Pennsylvania, found that *P. melanarius* contributed 3-73% of the total carabid species caught in the pitfall traps. In two cases it even played an important role in the control of invertebrate pests like slugs and caterpillars [17]. Even though seasonal changes in carabid communities are also common phenomena particularly in agricultural settings, it is interesting to note that this particular species is native to Europe and is therefore considered an invasive, nonetheless, it can make a significant impact on pest control on occasion. Despite the fact that most of the exotic predator species used in pest control were introduced on purpose in the context of traditional biological control, the vast majority of the exotic predator species used in pest control are intentionally introduced. Exotic species that are unintentionally introduced yet end up being useful in controlling economic pests are rare. Even more rare are exotic predators that not only do not cause any issues in and of themselves, but also help with pest management.

Use of insecticides by conventional farmers to prevent insect infestation often does little to help the farmer maintain the population of natural enemies [18-20]. Carbamates, pyrethroids and neonicotinoids are insecticides that are effective against carabid beetles. Insecticides pose a number of threats to carabids, including direct exposure, indirect exposure through eating infected food, and famine due to a lack of prey. When exposed to them at close quarters, insecticides have a deleterious impact on carabids. In farming fields, during which several field operations are undertaken, including the use of pesticides, the risk of adult mortality is increased due to the activity of *Pterostichus melanarius*. A topical pyrethroid treatment was most sensitive to Pterostichus melanarius among large carabid species in a laboratory environment with an LD50 of 135.8 ng arthropod-1. *P. melanarius* was 62% more prevalent in plots that got pyrethroid applications at a rate one-fourth of the recommended field rate as compared to full field application rates. The activity densities of all the carabid species were reduced by 90% instantly following an autumn pyrethroid activity in an open field, and the decrease persisted with a significantly lower activity density of *Pterostichus melanarius* 30 days after the application [21-23]. In the case of *Pterostichus melanarius*, the temporary reduction in the organophosphate rate in the field also resulted in a few weeks of a temporary reduction in the organophosphate rate in the field compared to non-treated plots. It may also happen that the actual effects of pesticides on populations of carabids are masked in open field experiments by the mobility and dispersal of beetles. There is also a roundabout route in which pesticides can impact carabids. *Pterostichus melanarius* will consume poisonous food at the expense of consuming insects that have been contaminated with pesticides. All aphids that were consumed by *Pterostichus melanarius* died following the consumption of an organophosphate killed [24]. The three-day mortality rate of feeding on aphids exposed even to a tenth of the field treatment rate was 90%. One of the eighteen carabid species that became extinct by feeding on maize rootworms which had eaten maize seeds treated with neonicotinoid pesticides. Another factor that can make carabids more vulnerable to prey scarcity and famine is the use of pesticides. The insecticides used in spring and fall temporarily reduced the Pterostichus melanarius activity density, which was followed by a large rebound 35 to 50 days later, exceeding control plot activity densities [25]. However, this activity density spike was presumably due to more foraging and less prey. Moreover, in one study, the gut content analysis indicated that the fish had fed more in the untreated plots than in the insecticide-treated plots, which gives credence to the notion that the fish had engaged in more foraging in the untreated plots as compared to the insecticide-treated plots [26-28]. Farmers should implement no-till methods and maintain a reasonably diverse rotation with cover crops to enhance the chances of Pterostichus melanarius and other carabid species making a significant contribution to pest control [29-31]. The best thing is that farmers can use insecticides within a framework of an integrated pest management system so that they do not harm *Pterostichus melanarius*.

CONCLUSIONS

Although *Pterostichus melanarius* is a predator and is highly important in some ecosystems. According to studies, *P. melanarius* can be effectively used to control insect and slug pests in many agricultural systems. It is recommended to use insecticides as part of integrated pest management to protect *Pterostichus melanarius*, a predatory fish, because of its sensitivity to them. Studies indicate that *Pterostichus melanarius* is most useful in no-till or reduced-till systems with a variety of rotations, like cover crops or intercropping. *Pterostichus melanarius* can potentially be applied in various agricultural practices, as the range extends. It is important to record its existence and see if agricultural systems may be adjusted to make *Pterostichus melanarius* a more effective pest controller. This research supports the entomo-ethno medical usage of ground beetle in the treatment of bacterial infections and proposes that the methanol extract of the ground beetle contains bioactive compounds such tannins, flavonoids and alkaloids which have an antifungal effect. The GC-MS characterization of the bioactive components has helped to identify some significant fragments and functional groups. The levels of metabolite activity in ground beetles (*Pterostichus melanarius*) were rather high against *Aspergillus flavus* (26.07±0.48).

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